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Ontogeny of macroscopic morphology of limb bones in modern aquatic birds and their implications for ontogenetic ageing

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Abstract: Although the assessment of ontogenetic stage of fossil and skeletal specimens (ontogenetic ageing) plays fundamental roles in avian paleontology, detailed information of postnatal ontogeny of macroscopic morphology, which is essential for ontogenetic ageing, has been lacking for the avian skeleton. In this study, the postnatal skeletal development was described for six major limb bones (humerus, ulna, carpometacarpus, femur, tibiotarsus, and tarsometatarsus) in four species of modern aquatic birds (*Calonectris leucomelas*, *Phalacrocorax capillatus*, *Larus crassirostris*, and *Cerorhinca monocerata*) from 14–28 individuals of known ontogenetic stages for each species, with emphasis on the macroscopic morphology and timing of skeletal maturity. The presence of the epiphysal ossification center at the proximal tibiotarsus was confirmed in the all species except *Larus crassirostris* where old chicks were poorly sampled. Epiphysal ossification centers may be variably observed in other limb bones. A distinct ossification center is observed in crista fibularis of the tibiotarsus in *Phalacrocorax capillatus*. Foramen nutrienis is generally larger in non-adults than in adults, and in some cases there may even be multiple foramina. Longitudinal growth of bones generally ceases around the time of fledging, by which epiphyses are ossified in most cases. Limb bones of fledglings/juveniles were generally as large as those of adults, but tend to be more slender, indicating that circumferential bone growth is still in progress in these stages. Although the overall pattern of morphological variation is consistent among the species examined, one should be aware of interspecific variation in the timing of skeletal maturity, as well as inter-elemental variation in the timing of ossification of epiphyses.

Key words: Skeletal ontogeny, Ossification center, Nutrient foramen.

Resumen: A pesar del rol fundamental que el estadio ontogenético de especímenes esqueléticos fósiles y actuales juega en la paleontología aviana, información detallada de la ontogenia postnatal de la morfología macroscópica, la cual es esencial para reconocer la edad del individuo, no se ha descrito para el esqueleto de las aves. En este estudio, el desarrollo esquelético postnatal es descrito para seis huesos principales (húmero, ulna, carpometacarpo, fémur, tibiotarso, y tarsometatarso) en cuatro especies de aves acuáticas modernas (*Calonectris leucomelas*, *Phalacrocorax capillatus*, *Larus crassirostris* y *Cerorhinca monocerata*) para 14–28 individuos de cada especie, cuyo estadio ontogenético es previamente conocido, con un énfasis en la morfología macroscópica y el tiempo de la madurez esquelética. La presencia de un centro de osificación epifisiario en el sector proximal del tibiotarso fue confirmado para todas las especies a excepción de *Larus crassirostris* donde los pichones viejos fueron pobremente muestreados. Centros de osificación epifisiarios son observados de manera variable en otros huesos de los miembros. Un centro de osificación bien diferenciable se observa en la cresta fibular del tibiotarso en *Phalacrocorax capillatus*. El foramen nutrienis es generalmente de mayor tamaño en los ejemplares no-adultos que en los adultos, y en algunos casos, pueden existir múltiples forámenes. El crecimiento longitudinal de los huesos generalmente culmina cerca del momento de adquirir plumaje, cuando las epifisis ya se encuentran osificadas. Los huesos de los miembros de juveniles fueron de tamaño semejante al de los adultos, pero tienden a ser más delgados, indicando que el crecimiento de hueso circunferencial se encuentra aún en progreso a esas edades. A pesar que el patrón de variación morfológico es consistente entre las especies examinadas, se debe estar consciente de la variación interespecífica en el tiempo de la madurez esquelética, así como de la variación inter-elementos en el momento de osificación de las epifisis.

Palabras clave: ontogenia esquelética, centro de osificación, foramen nutricio.

INTRODUCTION

Ontogenetic ageing, the assessment of ontogenetic age or stage of specimens, plays fundamental roles in paleontological and morphological studies. In general, correct ontogenetic ageing is necessary in taxonomic assignment of fossil specimens, which is essential for systematics, paleoecology, and evolutionary studies. In avian paleontology, an occurrence of immature individuals can be evidence of breeding of the species near the locality at the time of deposition, which is of potential importance in biogeography or paleoclimate (Matsuoka, 2000; Picasso *et al.*, 2011; De Pietri *et al.*, 2016) and in inferring seasonality in zooarchaeology (e.g., Howard, 1929; Serjeantson, 1998). Age structures of fossil assemblages may reveal breeding strategies of extinct animals (Turvey & Holdaway, 2005). Immature individuals and adults of seabirds may have different geographic sources of food items and hence isotopic compositions, which necessitates ontogenetic ageing of specimens in isotopic analyses (Wiley *et al.*, 2013). For such studies, reliable ontogenetic ageing criteria for (isolated) avian skeletal elements are required.

Ontogenetic ageing of fossil bones heavily depends on knowledge of skeletal ontogeny, and macroscopic morphological features can sometimes be a diagnostics for ontogenetic ageing. However, in contrast to the extensive literature on the embryonic development, information on the postnatal ontogeny of the avian skeleton is scarce, apart from metrical (e.g., Latimer, 1927; Cane, 1993; Hayward *et al.*, 2009; Picasso, 2012) or histological aspects (e.g., Chinsamy, 1995; de Margerie *et al.*, 2002, 2004). As a consequence, avian paleontologists have been forced to rely on relatively limited material of (supposedly) immature individuals when inferring ontogenetic variation (e.g., Mayr, 2005). Detailed knowledge of the avian postnatal skeletal ontogeny from large series of known-aged individuals is desired.

As a basis for ontogenetic ageing, Watanabe & Matsuoka (2013) described the postnatal ontogeny of macroscopic morphology of major limb bones in *Ardea cinerea* (grey heron) from 17 individuals of known ontogenetic stages (chick, juvenile, and adult). The present study aims to add some more knowledge on the avian skeletal ontogeny by preparing and examining ontogenetic series of four additional species of modern birds, *Calonectris leucomelas* (streaked shearwater, Procellariidae), *Phalacrocorax capillatus* (Japanese cormorant, Phalacrocoracidae), *Larus crassirostris* (black-tailed gull, Laridae), and *Cerorhinca monocerata* (rhinoceros auklet, Alcidae). Ontogenetic changes of macroscopic morphology and metric characters of six major limb bones (humerus, ulna, carpometacarpus, femur, tibiotarsus, and tarsometatarsus) are described and illustrated. Some features of morphological interests are also described, including ossification centers in femur and tibiotarsus. Associated results of examinations of bone surface texture and histological structure for textural ageing (Tumarkin-Deratzian *et al.*, 2006, 2007; Tumarkin-Deratzian, 2009) will appear elsewhere. (Watanabe, 2017).

MATERIALS AND METHODS

Target species and ontogenetic stages

In order to investigate the skeletal ontogeny of birds, materials of known ontogenetic stages were collected for four species of modern birds: *Calonectris leucomelas*, *Phalacrocorax capillatus*, *Larus crassirostris*, and *Cerorhinca monocerata*. An ontogenetic series of *Ardea cinerea* (gray heron, Ardeidae) described by Watanabe & Matsuoka (2013) was also reexamined. The target species were chosen for the ease of collecting a large number of immature individuals and the availability of ecological information.

Calonectris leucomelas is a large shearwater weighting about 440–545 g as adults (Carboneras, 1992). It is sexually dimorphic, with males being ~5% larger (up to 15% for bill depth) than females in external measurements (Shirai *et al.*, 2013; Arima *et al.*, 2014). Chicks are altricial and stay within their natal nest cavity for a prolonged period, being fed by parents. As in other procellariid species, weights of chicks generally reach a peak which exceeds the adult range well before fledging, after which parents cease to feed chicks and weights of chicks decrease (Oka *et al.*, 2002). The age at fledging is ~75 days in the studied population. *Phalacrocorax capillatus* is a large cormorant weighting about 2.5–3.5 kg as adults, with males being markedly larger than females in external measurements (Johnsgard, 1993). Chicks are altricial and stay around the natal nest. They leave the nest at ~40 days old (Yamamoto, 1967). *Larus crassirostris* is a medium-sized gull weighting ~500 g as adults, with males being slightly larger than females, especially in bill dimensions. Chicks are precocial and able to walk and run soon after hatching. They fly around the nest as early as ~35 days old, and leave the nest at ~45 days old. *Cerorhinca monocerata* is a medium-sized puffin weighting about 480–620 g as adults, with males being generally larger than females (Gaston & Jones, 1998). Chicks are semiprecocial and stay in the natal nest cavity, being fed by adults. Body mass of chicks may decline after the cessation of feeding by adults which takes place before fledging. There are large variations in size and age at fledging; fledglings weigh 51–88% of adult mass, and fledging occurs at 40–70 days old (on average ~50 days; Ydenberg, 1989; Deguchi *et al.*, 2004).

For each species, two or three of the following ontogenetic stages were recognized as distinct classes: the chick, fledgling or juvenile, and adult stages. The chick stage is defined as birds that are collected within natal colonies or a natal nest cavity and characterized by immature plumages including remiges partly enclosed in sheaths, and is recognized for all species. The fledgling stage, recognized for *Calonectris leucomelas* and *Cerorhinca monocerata*, is defined as birds that had just left their natal nests (see below). The juvenile stage, recognized for *Ardea cinerea* and *Phalacrocorax capillatus*, is defined as birds that can be distinguished from adults by pre-definitive plumages, including the gray head and neck in *Ardea cinerea* and the white underparts in *Phalacrocorax capillatus*. All juveniles examined in this study were collected in their first summer (i.e., ~2–5 months old). The adult stage, recognized for all species, is defined as birds with definitive plumages. For *Calonectris leucomelas*, *Larus crassirostris*, and *Cerorhinca monocerata*, all adults were collected in colonies as breeding adults. For *Ardea cinerea* and *Phalacrocorax capillatus*, adults were generally collected outside colonies, but in most cases their sexual maturity was confirmed by the development of gonads. For each species, 14–28 individuals were prepared as skeletal specimens that form an ontogenetic series.

Collection of materials

Material of *Calonectris leucomelas* was obtained at a breeding colony on Awashima Island, Niigata Prefecture, Japan, mainly during the breeding season of 2013. From late August to late October, growth of chicks was monitored in 28 predefined nests once in every five days. Ages of chicks at the initial monitoring were estimated from nonlinear regression of age to bill length (M. Shirai, personal communication). Seven chicks of known ages and five adults were captured. Captured individuals were euthanized with the inhalation of halothane. Four chicks and two adults, which had died from natural causes, were added to the ontogenetic series. Ten fledglings, which died during their first travel to the sea near the end of the breeding seasons of 2012 and 2014, were also used. The fieldwork was approved by the Ministry of Environment and Agency for Cultural Affairs (No. 10).

Material of *Ardea cinerea* was obtained as described in Watanabe & Matsuoka (2013). Exact ages of chicks were not known, and hatchlings were not available. The two juveniles were determined to be in their first summer (i.e. ~2–4 months old) from the plumage and collection dates. One additional adult which had died from a natural cause was also included.

Material of *Phalacrocorax capillatus* was mainly obtained on Teuri Island, Hokkaido Prefecture, Japan, during the breeding seasons of 2013 and 2014. Fifteen dead chicks were collected on the coast near a breeding site. Exact ages of chicks were unknown, and hatchlings were not available. Two adults which died from natural causes on the island were obtained. Two juveniles, which had been rescued near the island (thus probably from the same population), but subsequently died from natural causes, were provided by the Hokkaido Seabird Center, Haboro, Japan. The two juveniles were determined to be in their first summer (i.e. ~2–5 months old) from the plumage and collection dates. The fieldwork was approved by the Agency for Cultural Affairs (No. 24-4-2133).

Material of *Larus crassirostris* was obtained at a breeding colony on Rishiri Island, Hokkaido Prefecture, Japan, during the breeding season of 2014. From late May to late June, hatching dates and growth of chicks were monitored in a predefined study area once in every 1–4 days. One chick of known age and four breeding adults were captured, which were subsequently euthanized by halothane. Nine chicks of known ages which died from natural causes in the study site during the period were also included. Due to the poor environmental condition in the season, it was not possible to obtain old chicks and fledglings in the study season. The fieldwork was approved by the Hokkaido Soya Subprefectural Bureau (No. 13).

Material of *Cerorhinca monocerata* was obtained at a breeding colony on Teuri Island, Hokkaido Prefecture, Japan, during the breeding season of 2013. Hatching dates and growth of chicks were recorded in 18 predefined nests once in every five days. Eight chicks of known ages were captured to span most of the nesting period with intervals of five days. In the same breeding season, two fledglings were captured on the way from their natal nest to the sea. Captured individuals were euthanized by the inhalation of carbon dioxide after the anesthetization by isoflurane. Two additional fledglings of the same season and four adults which died from natural causes were also obtained. The fieldwork was approved by the local government of Hokkaido (No. 1293), the Agency for Cultural Affairs (No. 24-4-2133), and the Ministry of Environment (No. 1302151).

Preparation of specimens

For *Calonectris leucomelas* and *Cerorhinca monocerata*, dissection was conducted soon after euthanization, and carcasses were then kept frozen until further preparation, with skin, feathers, and major internal organs removed. For the other species, carcasses were frozen soon after collection. On further preparation, carcasses were thawed in room temperature and one or both sides of forelimbs and hind limbs were excised from the trunk. When possible, individuals were sexed by the visual inspection of gonads. Limb bones were prepared as dried specimens as described in Watanabe & Matsuoka (2013); bones were isolated by removing soft tissues, bleached with ~2% solution of hydrogen peroxide for 12–24 h, and dried at 50°C for 12–24 h. Remaining soft tissues and periosteum were removed as required. When available, left bones were preferred for observations.

In this study, each individual is referred to by an individual code, which consists of a code for species (CL for *Calonectris leucomelas*, PH for *Phalacrocorax capillatus*, LA for *Larus crassirostris*, and CM for *Cerorhinca monocerata*), code for ontogenetic stages (C for chick, F for fledgling, J for juvenile, and A for adult) and a number

within each combination of species and ontogenetic stages (e.g., CL-C1 refer to the first chick of *Calonectris leucomelas*). Numbers are not necessarily meant to represent ontogenetic sequences among individuals. Species codes are not shown when not ambiguous. Individuals used in this study are listed in Appendix A. For more detailed information, see Watanabe (2016). The ontogenetic series examined in this study are stored at the Department of Geology and Mineralogy, Kyoto University, Kyoto, Japan.

Observation and measurements

Macroscopic morphology was observed mainly in dried bones with naked eyes and a $\times 10$ hand lens. Descriptive terminology follows Baumel & Witmer (1993). Except for *Ardea cinerea*, osteological measurements were taken during preparation, after bleaching and before drying, in order to avoid deformations of cartilages caused by drying. Care was taken to minimize deformations due to specimen manipulation and measurement. Measurement was taken with a digital caliper (Mitsutoyo, Japan) to nearest 0.1 mm.

Definitions of measurements are as follows. Humerus, length, greatest length from caput humeri to condylus ventralis (not to processus flexorius); shaft width, dorsoventral width of shaft around the midpoint, measured as the smallest diameter in the dorsoventrally compressed humeri of *Calonectris leucomelas* and *Cerorhinca monocerata*. Ulna: length, greatest length between olecranon and condylus dorsalis; shaft width, dorsoventral width of shaft around the midpoint. Carpometacarpus: length, greatest length between the proximal margin of trochlea carpalis and facies articularis digitalis major; shaft width, dorsoventral width of shaft of os metacarpale majus around the midpoint. Femur: length, greatest length between trochanter femoris and condylus lateralis; shaft width, mediolateral width of shaft around the midpoint. Tibiotarsus: length, length between articular surfaces measured from facies articularis lateralis to the more distal one of distal condyles (condylus medialis in *Phalacrocorax capillatus*, condylus lateralis in the other species), measured parallel to the shaft; shaft width, mediolateral width at the narrowest point of distal shaft. Tarsometatarsus: length, greatest length from the proximal tip of the proximal articular surface to trochlea metatarsi III; shaft width, mediolateral width of shaft at the midpoint.

The differences of skeletal dimensions between the fledgling and adult stages were tested with the Kruskal-Wallis test implemented in R 3.3.1 (R core team, 2016). The statistical significance of multiple hypothesis testing was adjusted by controlling the false discovery rate (Benjamini & Hochberg, 1995).

RESULTS

Description

Considerable ontogenetic variation of macroscopic morphology was observed in the ontogenetic series examined. Morphological descriptions are given below with emphasis on ontogenetically variable features. See Watanabe & Matsuoka (2013) for results in *Ardea cinerea*.

Calonectris leucomelas

Humerus (Fig. 1A, B)

Chick—In young chicks (e.g., C1), the shaft is relatively thin and strongly convex dorsally. Caput humeri is cartilaginous in young chicks (up to ~50 days old, C1–C5) and gradually ossifies thereafter. Tuberculum ventrale is completely cartilaginous in young chicks (up to ~40 days old, C1–C4), partly ossified in ~50 days old chick

(C5), and mostly ossified in older chicks (C6, C7). The excavation below tuberculum ventrale occurs about the same time as the ossification of the tubercle, around ~50 days old (C5), and mostly complete in oldest chicks (e.g., C7). Tuberculum dorsale is formed as late as ~60 days old (C6). Crista deltopectoralis is a blunt crest with a cartilaginous tip in young chicks (C1–C5), and is mostly ossified in older chicks (~60 days old and older; C6, C7). The dorsal surface of crista deltopectoralis is flat until ~20 days old (C1, C2), weakly concave thereafter until ~50 days old (C3–C5), and as well ossified in older chicks (C6, C7). Linea m. latissimus dorsi is absent in young chicks (C1–C5) whereas it is present in the older chicks, but does not extend over the distal end of crista deltopectoralis until ~70 days old (C7). Foramen nutriens is always single and present as a large, distinct foramen on the ventral margin of the shaft (Fig. 1B). Condylus dorsalis et ventralis and epicondylus dorsalis et ventralis are cartilaginous in young chicks, whereas they are mostly ossified after ~60 days old (C6 and C7). There are numerous pores on the ossified distal condyles. Tuberculum supracondylare ventrale is present from ~40 days old (C4 and older); at first it is thin, but as large in the oldest chick (C7) as in adults. Processus supracondylaris dorsalis is completely cartilaginous until ~50 days old (C5), partly ossified in ~60 days old (C6), and almost completely ossified in ~70 days old (C7) (Fig. 1A). Fossa brachialis is not well marked in young chicks, and begins to develop from ~50 days old (C5).

Fledgling—Qualitative features of humeri in juveniles are mostly similar to those in adults. Caput humeri is ossified and entire, but surrounded by minute pores. Foramen nutriens is present on the ventral margin of the shaft, and its opening is distinctively larger than in adults (Fig. 1B). Extremitas distalis humeri is as well developed as in adults (Fig. 1A), but the surface of distal articular condyli and fossa olecrani is somewhat porous.

Adult—Caput humeri is ossified, proximally round, and with few pores on its surface. Tuberculum ventrale is relatively thick and protruding caudally. Tuberculum dorsale is clearly delineated. Crista deltopectoralis is completely ossified, triangular in dorsal view, and with a blunt tip pointing cranially. The dorsal surface of the crest is concave and delineated by a thick and distinct margin. Linea m. latissimus dorsi extends over the proximal one-fourth of the shaft. Fossa tricipitalis deeply excavates below tuberculum ventrale, interrupted by a sharp crus dorsale fossae. A single foramen nutriens is present on the ventral margin of the shaft at around one-third of the shaft from the proximal end, and its opening is relatively minute (Fig. 1B). Condylus dorsalis et ventralis and epicondylus dorsalis et ventralis, tuberculum supracondylare ventrale, and processus supracondylare dorsalis are completely ossified (Fig. 1A). Few pores are present on the surface of the distal condyles. The entire margin of fossa brachialis is relatively distinct.

Ulna (Fig. 1C)

Chick—Extremitas proximalis ulnae, including olecranon, is entirely cartilaginous in young chicks (C1–C5), and is ossified in chicks over ~60 days old (C6 and C7). Tuberculum lig. collateralis ventralis is not ossified before ~50 days old (C1–C4), and gradually develops thereafter. Impressio m. brachialis is ill-defined, and the corresponding part is flat to convex in young chicks (up to ~40 days old, C1–C4) and shallowly excavated in older chicks (C5–C7). A single foramen nutriens is present on the cranial margin of the midshaft with a large opening (Fig. 1C). Papillae remigales are absent, except for a few ones on the midshaft in the oldest chick (~70 days old, C7). Linea intermuscularis is indistinct. Extremitas distalis ulnae is completely cartilaginous in chicks up to ~50 days old (C1–C5), and is mostly ossified in older chicks (C6, C7).



Fig. 1. Ontogenetic variation of macroscopic morphology of *Calonectris leucomelas* forelimb bones. A, distal end of humerus, cranial view; B, midshaft of humerus, ventral view, showing foramen nutiens (white arrowheads); C, midshaft of ulna, cranial view, showing foramen nutiens (white arrowheads); D, proximal end of carpometacarpus, dorsal view, showing the sequence of ossification of carpal and metacarpal elements. Scale bar equals 1 cm. Labels below panels refer to individual codes in Table A1. Abbreviations: dca, the proximal os carpi distale; dcb, the distal os carpi distale; fmb, fossa musculi brachialis; mal, os metacarpale alulare; psd, processus supracondylaris dorsalis; tsv, tuberculum supracondylare ventrale.

Fledgling—Ulnae of fledglings are almost as well developed as in adults, and there are only a few qualitative differences. The opening of foramen nutrienis on the midshaft is much larger than in adults (Fig. 1C). Distal papillae remigales caudales are often indistinct, and 6–11 papillae are discernible. Papillae remigales ventrales are indistinct.

Adult—Extremitas proximalis ulnae, including olecranon, is ossified, and there are few pores on the articular surface. Tuberculum lig. collateralis ventralis is well elevated from the shaft, and a blunt ridge extends from the distal end of the tubercle. Impressio m. brachialis is deeply excavated. Foramen nutrienis is present on the cranial margin of the midshaft, with a minute opening (Fig. 1C). An array of 11 papillae remigales caudales are discernible on the caudal margin of the shaft, as well as five or six papillae remigales ventrales on the ventral margin of the midshaft. Linea intermuscularis is distinct on the cranial margin of the proximal half of the shaft. Extremitas distalis ulnae, including trochlea carpalis and tuberculum carpalis, is completely ossified.

Carpometacarpus (Fig. 1D)

Chick—Five distinct elements are recognized: one carpal forming a proximal part of trochlea carpalis (“semilunate” of Botelho *et al.*, 2014), another carpal forming a ventrodistal part of trochlea carpalis and processus pisiformis (“distal carpal 3” of Botelho *et al.*, 2014), ossa metacarpale alulare, majus, et minus (Fig. 1D). Ossa metacarpale majus, minus, et alulare are already ossified in the youngest individual (~10 days old, C1), the proximal carpal ossifies around ~30 days old (C3), and the distal carpal around ~40 days old (C4). They appear to begin to fuse with one another around ~50 days old (C5). A distinct suture is discernible between os metacarpale majus and the fused compound of os metacarpale alulare and the proximal carpus after the fusion, except in some oldest individuals (~75 days old, C11). Trochlea carpalis is mostly cartilaginous in youngest chicks (up to ~30 days old, C1–C3), but the proximal margin is formed by the carpi from ~30 days old (C3 and older). The tubercle for m. extensor carpi ulnaris is present in old chicks (from ~60 days old, C6) (Fig. 1D). Sulcus tendineus is present as a shallow furrow on the dorsocaudal margin of the shaft of os metacarpale majus from as early as ~20 days old (C2). Foramen nutrienis is present on the caudal margin of the shaft of os metacarpale majus with a relatively large opening. The distal articular surfaces are cartilaginous before ~60 days old (C6). When they are ossified, porosity on them is not prominent.

Fledgling—All elements are completely ossified and fused, with sutures between elements discernible only in some individuals (F5). Most qualitative features are quite similar to those in adults. Foramen nutrienis is slightly larger than those in adults.

Adult—All elements are completely fused with no trace of sutures. Trochlea carpalis is completely ossified. The tubercle for m. extensor carpi ulnaris is prominent on the dorsocaudal margin of os metacarpale majus just distal to the proximal symphysis. Sulcus tendineus is clearly marked throughout the caudodorsal margin of the shaft of os metacarpale majus. Foramen nutrienis is present on the caudal margin of os metacarpale majus with a minute opening. The distal articular surfaces are completely ossified.

Femur (Fig. 2A)

Chick—Both ends are cartilaginous in youngest chicks. Caput femoris is not ossified until ~30 days old (C3), ossified but relatively small until ~50 days old (C5), ossified and clearly elevated from collum femoris in older chicks (~60 days old and older, e.g., C6, C7). On surfaces of caput femoris and facies antitrochanterica, numerous

minute pores are present. Impressiones mm. et ligg. trochanteris are absent until ~30 days old (C3), present as indistinct depressions until ~50 days old (C5), and distinct in older ones. Linea intermuscularis cranialis is absent before ~40 days old (C4), and proximally distinct but fades around the midshaft in older individuals. Foramen nutriens is present at a variable position on the caudal margin of the shaft with a relatively large opening (Fig. 2A). Tuberculum m. gastrocnemialis lateralis is

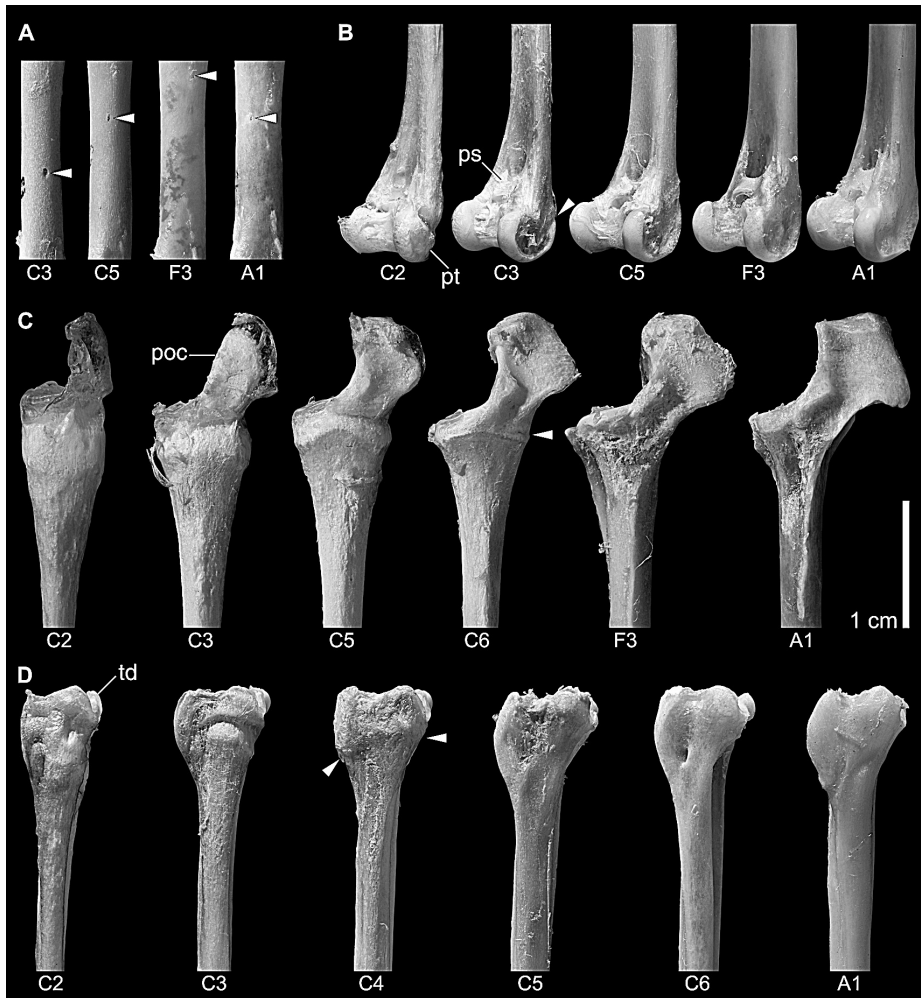


Fig. 2. Ontogenetic variation of macroscopic morphology of *Calonectris leucomelas* hind limb bones. A, midshaft of femur, caudal view, showing foramen nutriens (white arrowheads); B, distal end of tibiotarsus, craniolateral view, showing the fusion between the diaphysis and fused ossa proximalia tarsi with a transient suture (white arrowhead in C3); C, proximal end of tibiotarsus, medial view, showing the appearance and fusion of the proximal ossification center of tibiotarsus with a suture (white arrowhead in C6); D, proximal end of tarsometatarsus, medial view, showing the fusion between ossa tarsi distale and the fused shaft of metatarsi with a suture (white arrowheads in C4). Scale bar equals 1 cm. Labels below panels refer to individual codes in Table A1. Abbreviations: poc, proximal ossification center of tibiotarsus; ps, pons supratendineus; pt, fused ossa proximalia tarsi; td, os tarsi distale.

absent before ~30 days old (C3), present but indistinct until ~60 days old (C6), and well developed in oldest individuals (e.g., C7). *Extremitas distalis femoris* is at least partly cartilaginous until ~50 days old (C5), and ossified thereafter.

Fledgling—*Femora* of juveniles are qualitatively similar to those of adults. Exceptions are *foramen nutriens* which is slightly larger, and *linea intermuscularis cranialis* which is indistinct past the midshaft (Fig. 2A).

Adult—Both ends are completely ossified. *Caput femoris* is bulbous and distally elevated from *collum femoris*. Few pores are present on the proximal articular surface. *Linea intermuscularis cranialis* extends distally from the distal end of blunt *crista trochanterica*, bowed laterally around the midshaft to reach about two-thirds of the shaft from the proximal end. *Foramen nutriens* is present on the caudal margin of the shaft with a minute opening (Fig. 2A). *Tuberculum m. gastrocnemialis lateralis* is an irregular-shaped tubercle on the caudolateral margin of the distal shaft proximal to *condylus lateralis*. *Extremitas distalis femoris* is completely ossified with few pores on the articular surface.

Tibiotarsus (Fig. 2B, C)

Chick—The proximal end is disproportionately thick craniocaudally and tapers gradually from the proximal end. *Extremitas proximalis tibiotarsi* is entirely cartilaginous in youngest chicks (up to ~20 days old, C1, C2). An ossification center appears in the proximal epiphyseal cartilage at the position of *cristae cnemiales cranialis et lateralis* (Fig. 2C). It then extends caudally to cover the cranial half of the proximal articular surface in ~50 days old chick (C5), and fuses with the diaphysis before ~60 days old (C6). When present, the proximal ossification center forms *cristae cnemiales cranialis et lateralis*, which are not as well developed as in adults. The suture between the proximal ossification center and the diaphysis becomes obscure at ~70 days old (e.g., C7). In young chicks (up to ~30 days old, C1–C3), cranial and caudal margins of the proximal shaft are convex, and *fossa flexoria* is absent. In older chicks, the shaft tapers more steeply below the proximal end, and *fossa flexoria* is more or less concave. *Crista fibularis* is indistinct in chicks under ~60 days old (C1–C5), whereas it is well developed as a ridge on the lateral margin of the shaft in older chicks, which is slightly longitudinally shorter than in adults. *Foramen nutriens* is present on the medial margin of shaft caudal to fibula, just distal to the distal end of *crista fibularis* when present, and its opening is quite large and forms a small furrow in young chicks. *Extremitas distalis tibiotarsi* is cartilaginous in young chicks, within which the fused *ossa proximalia tarsi* are observed (Fig. 2B). *Ossa proximalia tarsi* first form the cranial part of distal condyles (C1, C2), then extend caudally and begin to fuse with the epiphysis in ~30 days old (C3). The suture between *ossa proximalia tarsi* and the diaphysis becomes indistinct as early as ~50 days old (C5). *Pons supratendineus* is at first a cartilaginous bridge formed between the diaphysis and *ossa proximalia tarsi*, and the ossification begins from the both sides from ~30 days old (C3; Fig. 2B). The ossification completes before ~60 days old (C6).

Fledgling—The overall profile is similar to that in adults. *Extremitas proximalis tibiotarsi* is ossified with no trace of suture. *Cristae cnemiales cranialis et lateralis* are ossified, but the cranial tip of *crista cnemialis cranialis* does not protrude cranially so strongly as in adults. *Crista fibularis* is well developed, but does not extend so distally in some individuals as in adults (Fig. 2C). *Foramen nutriens* is present around the distal end of *crista fibularis* and has a slightly larger opening than in adults, but does not form a furrow as in chicks. *Extremitas distalis tibiotarsi* is completely ossified with no trace of suture (Fig. 2B), but the articular surface of the condyles, especially the caudodistal part, is rather porous. *Pons supratendineus* is as well developed as in adults.

Adult—The shaft is almost in uniform width throughout the shaft. *Extremitas proximalis tibiotarsi* is ossified with no sign of suture. *Crista cnemialis cranialis* is well developed and protrudes strongly cranially in medial view. *Crista fibularis* is developed as a thin ridge. *Foramen nutriens* is present on the lateral margin of the shaft just caudal to the distal end of *crista fibularis*, and its opening is rather minute. *Extremitas distalis tibiotarsi* is ossified with no trace of suture.

Tarsometatarsus (Fig. 2D)

Chick—In young chicks (e.g., C1), the shaft is strongly tapered with both medial and lateral margins being concave in dorsal view, and the proximal part is wide mediolaterally. The proximal end is cartilaginous and contains a single ossification of *os tarsi distale* in young chicks (Fig. 2D). *Os tarsi distale* first forms the proximal articular surface (e.g., C1) and then extends caudally and distally to form the entire hypotarsus. It begins to fuse with the diaphysis of fused metatarsi ~40 days old (C4), and a trace of the suture remains discernible in some older chicks (~75 days old, C11). Margins of *sulcus extensorius* are rather blunt in young chicks, and *arcus extensorius* is not ossified. In young chicks (up to ~40 days, C1–C4), sutures between metatarsi are discernible in proximal and distal parts of the shaft. *Tuberositas m. tibialis cranialis* is present only in oldest individuals (e.g., C7), although indistinct. *Trochleae metatarsorum* are partly cartilaginous in young chicks but mostly ossified before ~40 days old (C4).

Fledgling—*Tarsometatarsi* of juveniles are qualitatively similar to those of adults. One exception is the absence (incomplete ossification) of *arcus extensorius*, for which a separate ossification is present in some individuals.

Adult—The shaft is straight and of uniform width. *Extremitas proximalis tarsometatarsi*, including hypotarsus, is completely ossified with no trace of suture. Margins of *sulcus extensorius* are well marked by distinct ridges. *Tuberositas m. tibialis cranialis* is present as a pair of small but distinct tubercles. *Arcus extensorius* is present on the medial margin of the proximal part of *sulcus extensorius* just above *tuberositas m. tibialis cranialis*. *Trochleae metatarsorum* are completely ossified.

Phalacrocorax capillatus

Humerus (Fig. 3A, B)

Chick—*Caput humeri* is entirely cartilaginous in small chicks, and ossified in a few largest individuals (e.g., C4, C9, C15; Fig. 3A). Even when ossified, it has a rather porous surface. *Fossa tricipitalis* is not developed in most individuals, where the corresponding part is convex or flat rather than concave (Fig. 3A). In largest individuals (C4, C9, C15), it is concave and developed as a fossa below *tuberculum ventrale*, but relatively shallow and not clearly defined. *Crus dorsale fossae* is thin and not clearly defined as in adults. In most individuals, *crista deltopectoralis* is not well developed and there is no concavity dorsal to it. In largest individuals (C4, C9, C15), the tip of *crista deltopectoralis* is thickened and deflected dorsocaudally, but not so strongly as in adults (Fig. 3A). The tubercle for the insertion of *m. scapulohumeralis cranialis* is discernible only in a few largest individuals (C9, C15), and *linea m. latissimus dorsi* is indiscernible except in C15. *Foramen nutriens* lies on the ventral margin of the shaft, in most cases (C11, C13, C7, C5, C4, C15) single, and lying slightly proximal to the midpoint of the shaft. In a few individuals (C1, C9), there is an additional *foramen nutriens* just distal to the one in the usual position (Fig. 3B). There does not seem to be a clear ontogenetic polarity for the number of *foramina nutrientia*. *Fossa m. brachialis* is not developed except in a few largest individuals (C4, C9, C15), where it is relatively shallow and has quite a rough surface texture. *Tuberculum supracondylare ventrale* is absent except in largest individuals

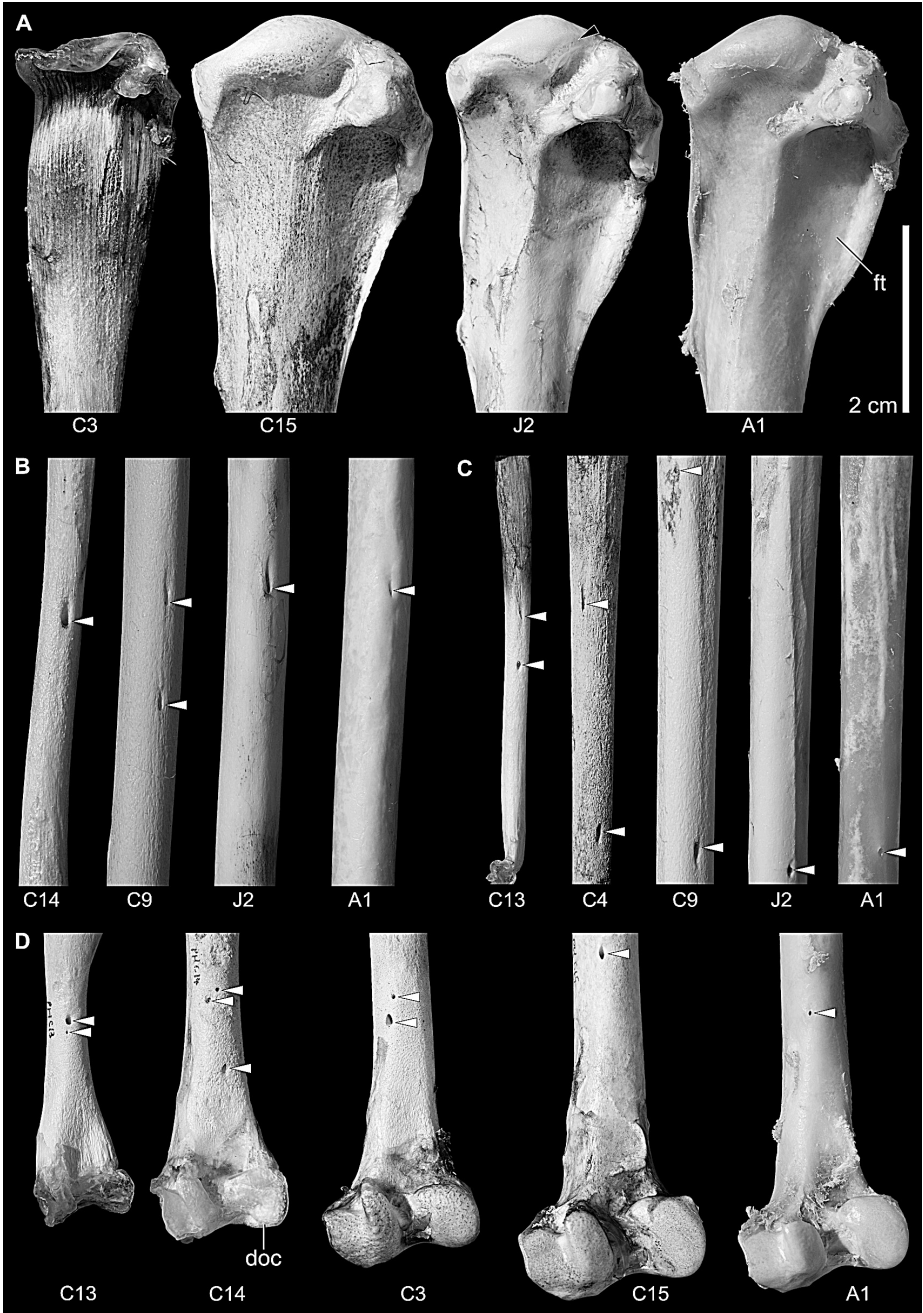


Fig. 3. Ontogenetic variation of macroscopic morphology of *Phalacrocorax capillatus* limb bones. A, proximal end of humerus, caudal view, showing the presence of porous area on the distal margin of caput humeri in juvenile (black arrowhead); B, midshaft of humerus, ventral view; C, midshaft of ulna, cranial view; D, distal part of femur, caudal view. Foramina nutrientia are indicated by white arrowheads. Scale bar equals 2 cm. Labels below panels refer to individual codes in Table A2. Abbreviation: doc, distal ossification center; ft, fossa tricipitalis.

(C4, C9, C15). Condylus dorsalis et ventralis are cartilaginous in most cases, and ossified in a few largest individuals (C4, C9, C15) but their surfaces are rather porous. Minute pores are also prevalent around fossa olecrani. The ventral margin of sulcus scapulothoracalis is not well defined except in a few largest individuals (C9, C15).

Juvenile—Humeri of juveniles are qualitatively similar to those of adults. Caput humeri is ossified and entire, but surrounded by numerous pores on its margins, especially on the caudal side (Fig. 3A). Incisura capitis also hosts numerous pores. Insertion for m. scapulohumeralis cranialis is relatively indistinct compared to that in adults. Foramen nutiens has a much larger and more distinct opening than in adults (Fig. 3B). Condylus dorsalis et ventralis are ossified, but, especially the latter, are surrounded by minute pores on their margins.

Adult—Caput humeri is completely ossified, and there are few pores on its surface. Incisura capitis hosts only a few pores (Fig. 3A). Crus dorsale fossae is thick but sharply defined, distal to which a round depression for the insertion of m. scapulohumeralis cranialis lies. The tip of crista deltopectoralis is thick and deflected dorsocaudally to form a concavity on its dorsal surface. Crista bicipitalis is also thick and its distal end lies as distal as the midpoint of impressio m. pectoralis. Impressio coracobrachialis is deep and its margins are clearly defined. The tubercle for m. latissimus dorsi caudalis is distinct, from which linea m. latissimus dorsi extends distally to reach ~40% of the shaft length from the proximal end. Foramen nutiens lies on the ventral margin of the shaft slightly proximal to the midpoint, with a single, minute opening (Fig. 3B). Fossa m. brachialis is relatively deep and clearly defined. Tuberculum supracondylare ventrale is distinct. Condylus dorsalis et ventralis are completely ossified and there are few pores on their surfaces. Margins of sulcus scapulothoracalis are defined by distinct ridges.

Ulna (Fig. 3C)

Chick—The craniocaudal curvature of the shaft is relatively weak in small chicks. Extremitas proximalis ulnae, including olecranon, is cartilaginous in small chicks, and mostly ossified in a few largest ones (C4, C9, C15). The articular surface is rather porous. In C4, the protrusion of the dorsocranial tip of cotyla dorsalis is not ossified, whereas it is in C9 and C15. In small chicks, tuberculum lig. collateralis ventralis is indiscernible, and impressio m. brachialis is indistinct with the corresponding and surrounding areas being convex rather than concave. In largest chicks (C4, C9, C15), these areas are concave with a distinct ridge in between, but impressio m. brachialis is ill-defined distally. A foramen nutiens is present on the cranial margin of the midshaft with a large opening (Fig. 3C). In most individuals except for the smallest (C13) and largest ones (C15), there is an additional foramen proximovertebral to the usual one, with a distinctive opening. In C13 and C14, there appears to be another foramen between them and near the dorsal margin of the bone. Papillae remigales caudales are quite indistinct, except for a few ones on the midshaft in largest chicks (C9, C15) where up to nine papillae are discernible. Papillae remigales ventrales are more indistinct, and only a few ones are discernible in the largest chick (C15). Linea intermuscularis is indistinct. Extremitas distalis ulnae is cartilaginous in small chicks, and is mostly ossified in oldest chicks (C4, C9, C15).

Juvenile—Ulnae of juveniles are qualitatively similar to those in adults with the following differences. The proximal articular surface is slightly porous. The openings of foramina nutientia are much larger than in adults (there is a single foramen nutiens in J2, and there are two in J1; Fig. 3C). The distal articular surface is slightly porous.

Adult—Extremitas proximalis ulnae is ossified, and there are few pores on the articular surface. The dorsodistal margin of cotyla dorsalis is protruding distally to give a hook-like appearance in dorsal view. Tuberculum lig. collateralis ventralis is

a distinct rugosity elevated from the shaft which overhangs on the proximoventral part of impressio m. brachialis. A blunt ridge extends from the distal end of the tubercle, which forms the ventral margin of impressio m. brachialis. A single foramen nutriens is present on the cranial margin of the midshaft, with a minute opening (Fig. 3C). In one individual (A2), but not in the other (A1), there is another foramen nutriens proximal to it with a rather minute opening. At least 11 papillae remigales caudales are discernible on the caudal margin of the shaft, as well as seven or more papillae remigales ventralis on the midshaft ventral to them. Linea intermuscularis is distinct on the cranial margin of the midshaft. Extremitas distalis ulnae is completely ossified.

Carpometacarpus

Chick—Five distinct elements are observed, as in *Calonectris leucomelas*. Ossa metacarpale majus et minus are ossified in all the chicks observed, os metacarpale alulare is ossified in all individuals except smallest ones (C11, C13), and the proximal and distal carpi are present in C3 and C5, but the latter is indistinct in both individuals. They are fused in largest chicks observed (C4, C9, C15), where sutures are more or less distinct. In C4, narrow fossae are present in some sutures, including that between os metacarpale alulae and os metacarpale majus, and between the fused carpi and os metacarpale majus (within fovea carpalis caudalis). Trochlea carpalis is mostly cartilaginous except in the largest individuals. The tubercle for m. extensor carpi ulnaris is present in large chicks (C4, C9, C15, C3, although indistinct in the last). In some largest individuals (C9, C15), sulcus tendineus is present as an indistinct furrow on the dorsocaudal margin of the distal end of os metacarpale majus. The number of foramina nutriens is variable among individuals. In most individuals, there is one foramen on each of cranial and caudal margins of os metacarpale majus, with a distinctive opening. Some individuals (C9, C14) lack the cranial one. One individual (C3) has an additional foramen on the cranial margin. The distal articular surfaces are cartilaginous except in largest individuals (C4, C9, C15) where the articular surfaces are rather porous.

Juvenile—Carpometacarpi of juveniles are qualitatively similar to those in adults. The only noticeable exception is the distinctively large size of foramen nutriens. In the two individuals available, foramen nutriens is present only on the caudal margin of os metacarpale majus.

Adult—All elements are completely fused with no trace of sutures. Trochlea carpalis is completely ossified. The tubercle for m. extensor carpi ulnaris and sulcus extensorius are prominent on the dorsocaudal margin of the proximal and distal parts, respectively, of os metacarpale majus. In A1, foramen nutriens is present on the cranial margin of os metacarpale majus, whereas in A2 it is present on the caudal margin. In any case, it has a minute opening. The distal articular surfaces are completely ossified.

Femur (Fig. 3D)

Chick—Extremitas proximalis femoris is cartilaginous in small chicks, and ossified in largest chicks (C4, C9, C15). In C5, caput femoris is partly cartilaginous but trochanter femoris is mostly ossified. In any case, numerous pores are present on the ossified proximal articular surface. Impressiones mm. et ligg. trochantericus and linea intermuscularis cranialis are indistinct in small chicks whereas they are as clearly marked in largest individuals (C4, C9, C15) as in adults. There are generally two or more foramina nutriens on the caudal margin of the shaft (Fig. 3D); in small individuals (C13, C11, C10, C12, C14, C6, C7, C2), there are three distinct foramina, two of which lie on the caudal margin around the midshaft and another lies more

distomedially, as well as occasional minute foramina around them. In some larger individuals (C3, C5, C4), only the two on the midshaft are distinct. In some largest individuals (C9, C15), there is only one large foramen on the midshaft. Most of these foramina are much larger than those in adults. Tuberculum m. gastrocnemialis lateralis is indiscernible in most individuals except for largest ones (C4, C9, C15). *Extremitas distalis femoris* is cartilaginous in small chicks. In some medium-sized chicks (C14, C2), an ossification center is present in the distal epiphyseal cartilage, which appears to fuse with the diaphysis later (Fig. 3D). The suture between the distal ossification center and the diaphysis is distinct in C3, C5, and C4, whereas indistinct in largest individuals (C9, C15).

Juvenile—Femora of juveniles are qualitatively similar to those of adults, but differ in the following points. There are two foramina nutrientia at variable positions on the caudal margin of the midshaft. Numerous minute foramina are present in fossa poplitea and the area proximal to it.

Adult—Both ends are ossified. Caput femoris is bulbous and with few pores on its surface. Impressiones mm. et ligg. trochanteris are well developed as several distinct depressions on the surface lateral to trochanter femoris. Linea intermuscularis cranialis extends from the distal end of crista trochanterica and over the midpoint. Foramen nutiens is present on the caudal margin of the midshaft, with a minute opening (Fig. 3D). Tuberculum m. gastrocnemialis lateralis is present as a rugose scar on the lateral margin of the shaft proximal to condylus lateralis. *Extremitas distalis femoris* is completely ossified with few pores on the articular condyles (Fig. 3D).

Tibiotarsus (Fig. 4A–C)

Chick—In small chicks, the proximal shaft is craniocaudally deep, and the distal shaft is strongly flared distally and wider than the distal end. *Extremitas proximalis tibiotarsi* is cartilaginous in small chicks. The proximal epiphyseal cartilage contains an ossification center in some large chicks (C3, C5) which forms cristae cnemiales cranialis et lateralis and the proximal articular surface (Fig. 4A). The proximal ossification center is fused with the diaphysis in largest chicks (C4, C9, C15), the suture between which is clearly demarked in C4 (Fig. 4A). Even when ossified, the proximal articular surface is rather porous. Cristae cnemiales cranialis et lateralis are cartilaginous in small chicks, formed by the proximal ossification center and rather indistinct in C3 and C5, and formed in the fused proximal end in largest chicks (C4, C9, C15). Even in the last case, the two crests are relatively blunt and the area between them is flat rather than concave as in adults. The proximal shaft is oval in cross-section and fossa flexoria is absent in small chicks. Fossa flexoria is concave in some large chicks (C4, C9, C15). Crista fibularis is indistinct in small chicks, whereas it is formed by a distinct ossification center in large chicks (Fig. 4B). The ossification center is triangular in cranial view and forms the distal part of crista fibularis. It then fuses with the diaphysis, leaving a prominent suture in largest chicks (C4, C9, C15). One or two foramina nutrientia is present at a comparable position as in adults, but is much larger. Another set of foramina nutrientia may be present in small chicks (e.g., C14) on the lateral margin of the distal shaft, around the distal attachment of the fibular ligament. *Extremitas distalis tibiotarsi* is cartilaginous in small chicks. In all individuals, the fused ossa proximalia tarsi are observed within the distal epiphyseal cartilage (Fig. 4C). They are fusing with the diaphysis in C5 and C3, and are completely fused in largest individuals (C4, C9, C11) with almost no trace of sutures. The ascending process of the tarsi is relatively short and has a round tip. Pons supratendineus is a cartilaginous bridge between the ascending process and the diaphysis of the shaft in small chicks. The ossification of the bridge begins from

both sides in C3 and C5, and is almost completed in largest chicks (C4, C9, C15).

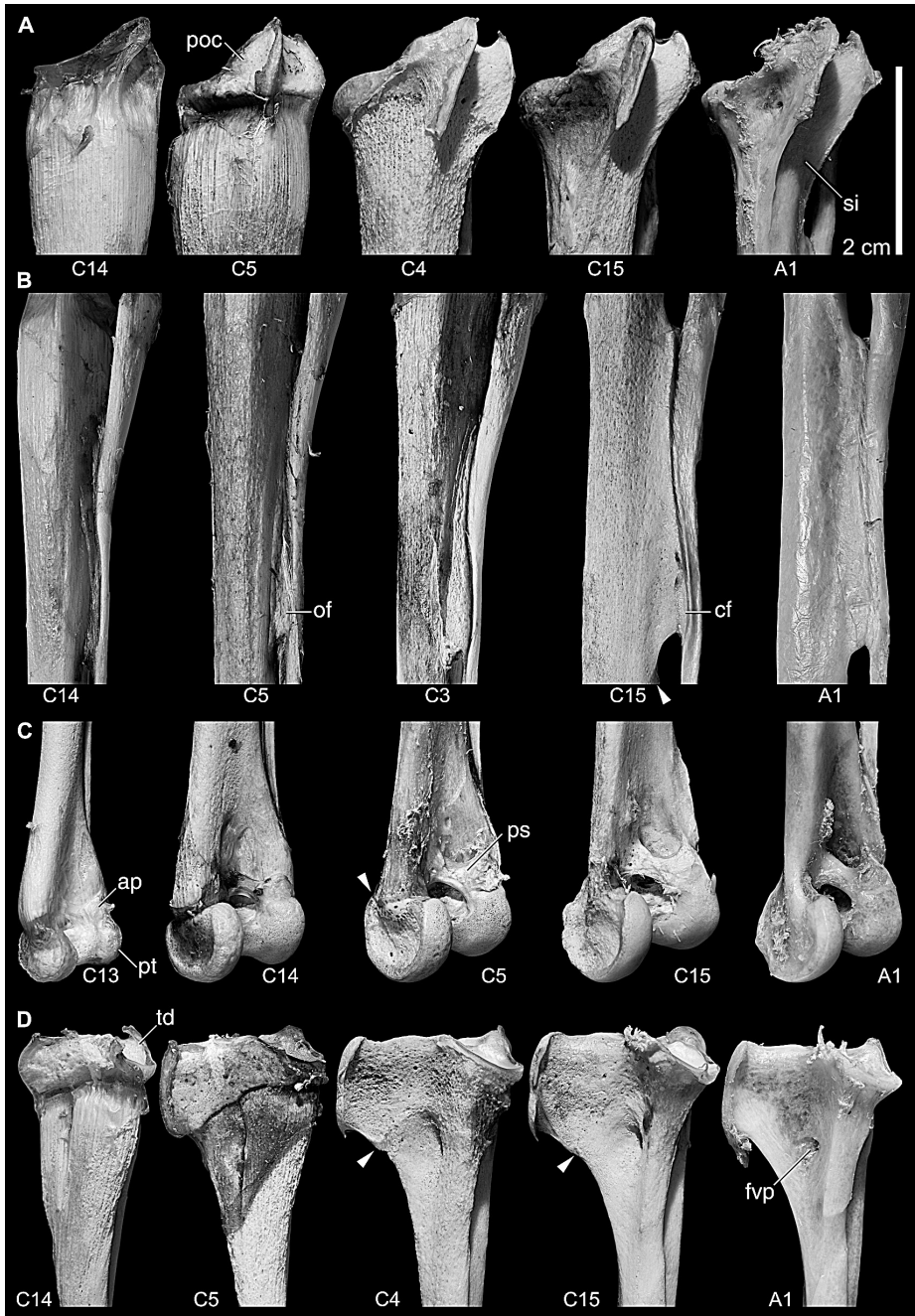
Juvenile—Tibiotarsi of juveniles are qualitatively similar to those in adults. There are minute pores on the proximal articular surface. Cristae cnemiales cranialis et lateralis are not as well developed as in adults, and their distal extents are not so sharp. Foramen nutriens is slightly larger than in that in adults.

Adult—The shaft is relatively uniform in mediolateral width and craniocaudal depth. Extremitas proximalis tibiotarsi is completely ossified with no trace of suture (Fig. 4A). There are few pores on the proximal articular surface. Crista cnemialis cranialis extends distally to form a sharp ridge on the craniomedial margin of the shaft which reaches one-fifth of the shaft length from the proximal end. Crista cnemialis lateralis also forms a sharp ridge on its distal extent, and the area medial to the ridge is slightly concave. Fossa flexoria is concave and is bounded medially by a ridge. Crista fibularis is a distinct ridge expanded strongly from the lateral margin of the shaft. There is no trace of suture on the base of the crest (Fig. 4B). Foramen nutriens is present on the laterocaudal margin of the shaft just distal to the distal end of crista fibularis, and with quite a minute opening. Extremitas distalis tibiotarsi is completely ossified with no trace of suture (Fig. 4C).

Tarsometatarsus (Fig. 4D)

Chick—In small chicks, the proximal shaft is flaring proximally and cross-section of the shaft is not strongly angular. In small chicks, extremitas proximalis tarsometatarsi is cartilaginous and contains os tarsi distale as a single ossification. The ossification of os tarsi distale forms only the proximal articular surface in small chicks, but in larger chicks it is extended caudally to form hypotarsus (Fig. 4D). Os tarsi distale and the diaphysis of the fused metatarsi are fused to each other in largest chicks (C4, C9, C15), where a distinct trace of suture is discernible. The lateral roof of the canal for the tendon of m. flexor digitorum longus (Mayr, 2016) is not ossified in small chicks, partly ossified in C4, and almost completely ossified in C9 and C15. Sulcus extensorius is ill-defined. Sutures between metatarsi are distinct on both the dorsal and plantar surfaces throughout the shaft in small chicks, but are indiscernible in largest chicks (C4, C9, C15). Foramina vascularia proximalia are formed within proximal gaps between unfused metatarsi and extremely long proximodistally in small chicks, and even in largest chicks they are much longer and larger than those in adults (Fig. 4D). Tuberositas m. tibialis cranialis is indiscernible except in C15 where it is rather indistinct. The sulcus on the medial surface of the proximal shaft is present in large chicks (C4, C9, C15), but not as clearly defined as in adults. Fossa metatarsi I is present as a indistinct depression on the medioplantar margin of the shaft even in smallest chicks (e.g., C13). Foramen vasculare distale is not closed distally in small chicks, and closed by incompletely formed distal walls in largest chicks (C4, C9, C15). Trochleae metatarsorum are cartilaginous in small

Fig. 4. Ontogenetic variation of macroscopic morphology of *Phalacrocorax capillatus* limb bones. A, proximal end of tibiotarsus, craniomedial view, showing the fusion of the proximal ossification center; B, midshaft of tibiotarsus, craniolateral view, showing the fusion of the ossification center for crista fibularis; C, distal end of tibiotarsus, craniomedial view, showing the fusion between the diaphysis and fused ossa proximalia tarsi; D, proximal end of tarsometatarsus, medial view, showing the fusion between os tarsi distale and the fused shaft of metatarsi. Positions of sutures are indicated by white arrowheads. Scale bar equals 2 cm. Labels below panels refer to individual codes in Table A2. Abbreviation: ap, ascending process; cf, crista fibularis; fvp, foramen vasculare proximale; of, ossification center for crista fibularis; poc, proximal ossification center; ps, pons supratendineus; pt, ossa proximalia tarsi; si, sulcus intercnemialis; td, os tarsi distale.



chicks and largely ossified in large ones (C5, C4, C9, and C15). The plantomedial tip of trochlea metatarsi II is developed only in C9 and C15. The surfaces of the trochleae are rather porous except in some largest chicks (C9, C15).

Juvenile—Tarsometatarsi of juveniles are qualitatively similar to those in adults.

But unlike adults, there are numerous minute foramina in fossa extensoria proximal to foramina vascularia proximalia.

Adult—The shaft is relatively uniform in mediolateral width and flaring steeply to the proximal and distal ends. *Extremitas proximalis tarsometatarsi* is completely ossified without any trace of suture (Fig. 4D). The canal for the tendon of *m. flexor digitorum longus* (Mayr, 2016) is enclosed by an ossified wall. *Sulcus extensorius* is deep and clearly defined by ridges on both medial and lateral margins of the shaft, the former of which is interrupted by a distinct sulcus on the medial surface of the shaft distal to *hypotarsus*. Sutures between metatarsi are indiscernible. *Foramina vascularia proximalia* lie on the bottom of *sulcus extensorius* and are relatively small, proximal to which another minute foramen is present. A distinct paired scar of *tuberositas m. tibialis cranialis* is situated distal to the foramina. *Foramen vasculare distale* is distally enclosed by two entire bone walls. *Trochleae metatarsorum* are completely ossified and there are few pores on their surfaces.

Larus crassirostris

Humerus (Fig. 5A)

Chick—Due to poor sampling, old chicks are not available for this species. In young chicks, the shaft is strongly tapered and convex dorsally. *Caput humeri*, *incisura capitis*, and *tubercula dorsalis et ventralis* are all cartilaginous in young chicks (at least until 19 days old, C1). *Crista deltopectoralis* is recognizable as an indistinct ridge on the dorsocranial margin of the proximal shaft just after hatching (e.g., C3), and remain unossified at least until 19 days old (C1). *Linea m. latisimi dorsi* is absent in all individuals observed. *Crista bicipitalis* is not ossified. The area corresponding to *fossa tricipitalis* is flat or convex in young chicks, but it is slightly concave in 19 days old chick (C1). A single *foramen nutriens* is present on the ventral margin of shaft slightly proximal to the midpoint, and with a large opening (Fig. 5A). *Condyli dorsalis et ventralis* and *epicondyli dorsalis et ventralis* are cartilaginous. The cranial surface of the shaft just proximal to the distal end is slightly concave in chicks of ~10 days old and older (C3, C1), but *fossa m. brachialis* is not well defined. *Tuberculum supracondylare ventrale* is not discernible. *Processus supracondylare dorsale* is cartilaginous.

Adult—*Extremitas proximalis humeri* is completely ossified. *Caput humeri* is bulbous, distally excavated by the dorsal part of *fossa tricipitalis*, and surrounded by few pores. *Linea m. latissimus dorsi* is distinct and reaches about one-third from the proximal end. A single *foramen nutriens* is present on the ventral margin of the shaft proximal to the midpoint, with a minute opening (Fig. 5A). *Condyli dorsalis et ventralis*, as well as *epicondyli dorsalis et ventralis*, are ossified and there are few pores on their surfaces. *Processus supracondylaris dorsalis* is ossified.

Ulna

Chick—In young chicks, the shaft is thin and convex caudally. *Extremitas proximalis ulnae*, including *olecranon*, is completely cartilaginous in all chicks observed (up to 19 days old, C1). *Tuberculum lig. collateralis ventralis* and *impressio m. brachialis* are indistinct, and the cranial surface of the proximal shaft is roughly convex. *Foramen nutriens* is present on the cranial margin of the shaft slightly proximal to the midpoint, with a distinct opening. *Papillae remigales* are not discernible as ossified structures. *Linea intermuscularis* on the cranial margin of the proximal shaft is indiscernible. *Extremitas distalis ulnae* is cartilaginous.

Adult—The shaft is relatively uniform in thickness throughout the shaft. *Extremitas proximalis ulnae*, including *olecranon*, is completely ossified, and there are few pores on the proximal articular surface. *Tuberculum lig. collateralis ventralis* is

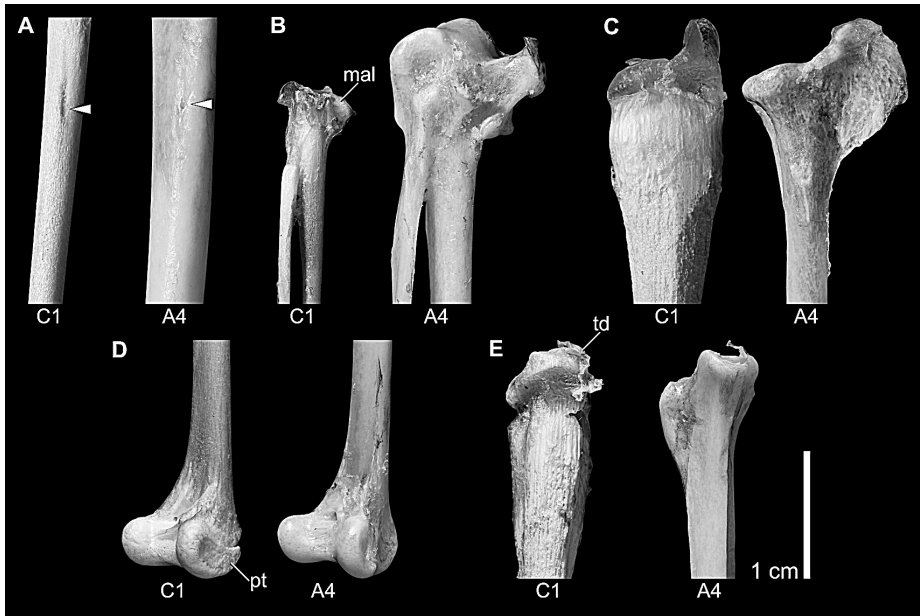


Fig. 5. Ontogenetic variation of macroscopic morphology of *Larus crassirostris* limb bones. A, midshaft of humerus, ventral view, showing foramen nutriens (white arrowheads); B, proximal end of carpometacarpus, dorsal view, showing the ossification of metacarpal elements; C, proximal end of tibiotarsus, medial view; D, distal end of tibiotarsus, craniolateral view, showing the ossification of ossa proximalia tarsi; E, proximal end of tarsometatarsus, medial view, showing the ossification of os tarsi distale. Scale bar equals 1 cm. Labels below panels refer to individual codes in Table A3. Abbreviations: mal, os metacarpale alulare; pt, fused ossa proximalia tarsi; td, os tarsi distale.

well elevated from the surrounding shaft and impressio m. brachialis is narrow but distinct. Foramen nutriens is present on the cranial margin of the shaft proximal to the midpoint. There are 13 or more papillae remigales caudales on the caudal margin of the shaft, and a comparable number of papillae remigales ventrales are also present ventral to them. Linea intermuscularis is distinct on the cranial margin of the proximal half of the shaft. Extremitas distalis ulnae is ossified.

Carpometacarpus (Fig. 5B)

Chick—At least three distinct elements are observed: ossa metacarpale alulare, majus, et minus. The two carpi observed in other species are not discernible at least until 19 days old (C1; Fig. 5B). Ossa metacarpale majus et minus are already ossified in the youngest individual, and os metacarpale alulare appears somewhere between 11 days old (C3) and 19 days old (C1). Timing of the fusions was not observable. Trochlea carpalis is completely cartilaginous in all individuals. Most osteological landmarks on the shaft, including the tubercle for m. extensor carpi ulnaris and sulcus tendineus, are indiscernible. Foramen nutriens is present on the caudal margin of the shaft of os metacarpale majus with a distinct opening. The distal articular surfaces are cartilaginous.

Adult—All elements are fused without any trace of sutures (Fig. 5B). Trochlea carpalis and processus alulare are ossified. The tubercle for m. extensor carpi ulnaris is present on the dorsocaudal margin of os metacarpale majus just distal

to symphysis metacarpalis proximalis. Sulcus tendinosus is distinct on the dorsal margin of the distal shaft of os metacarpale majus. Foramen nutiens is present on the caudal surface of the midshaft of os metacarpale majus, with a minute opening. The distal articular surfaces are completely ossified.

Femur

Chick—In young chicks, the shaft is strongly tapered and the distal end is relatively broad. Both ends are cartilaginous in all individuals available (up to 19 days old). Caput femoris is little more than a medial extension of the proximal shaft. Impressiones mm. et ligg. trochanteris and linea intermuscularis cranialis are indiscernible. Foramen nutiens is present on the caudal margin of the midshaft with a relatively large opening. Tuberculum m. gastrocnemialis lateralis is indiscernible. Extremitas distalis femoris is cartilaginous.

Adult—The shaft is relatively uniform in thickness. Caput femoris and trochanter femoris are completely ossified. Impressiones mm. et ligg. trochanteris are present as small but distinct tubercles or pits on the lateral surface of trochanter femoris. Linea intermuscularis cranialis extends distally from the proximal end medial to crista trochanterica and over the midpoint of the shaft. Foramen nutiens is present on the caudal margin of the shaft around or proximal to the midpoint of the shaft, with a minute opening. Tuberculum m. gastrocnemialis lateralis is small but distinct and lies on the caudolateral margin of the shaft just proximal to condylus lateralis. Extremitas distalis femoris is completely ossified.

Tibiotarsus (Fig. 5C, D)

Chick—The proximal end is disproportionately thick craniocaudally and the distal end is wide mediolaterally in young chicks. Extremitas proximalis tibiotarsi is cartilaginous in all available individuals, and the proximal ossification center observed in other species is indiscernible at least until 19 days old (Fig. 5C). Cristae cnemiales cranialis et lateralis are almost entirely cartilaginous. Cranial and caudal margins of the proximal shaft are convex rather than concave. Crista fibularis is indistinct. Foramen nutiens is present on the lateral margin of the shaft caudal to fibula, with a large opening which forms a small fossa in some individuals. Extremitas distalis tibiotarsi is cartilaginous and contains the ossification of ossa proximalia tarsi. In chicks up to 6 days old (e.g., C7), only the ascending process of the tarsi is ossified. In ~10 days old chicks, ossa proximalia tarsi form the cranial part of distal condyles, whereas they cover the entire distal end in 19 days old chick (C1; Fig. 5D).

Adult—The shaft is relatively uniform in thickness, and the proximal end flares abruptly below the articular surface. Extremitas proximalis tibiotarsi, including cristae cnemiales cranialis et lateralis, is completely ossified (Fig. 5C). The area between the distal extent of crista cnemialis cranialis and crista fibularis is flat. Fossa flexoria is concave. Crista fibularis is well developed on the lateral margin of the proximal shaft. Foramen nutiens is present on the lateral part of the shaft just distocaudal to the distal end of crista fibularis, and its opening is very minute but may be extended proximally. Extremitas distalis tibiotarsi is completely ossified with no trace of suture (Fig. 5D).

Tarsometatarsus (Fig. 5E)

Chick—In young chicks, the proximal shaft is disproportionately wide mediolaterally. Extremitas proximalis tarsometatarsi is cartilaginous. Os tarsi distale begins to ossify in the proximal epiphyseal cartilage around eminentia intercondylaris in 7–10 days old chick (C10). In 19 days old chick (C1), the entire articular surface and a part of hypotarsus is covered by os tarsi distale (Fig. 5E). Sulcus extensorius

is broad and ill-defined. Tuberositas m. tibialis cranialis is indiscernible. Sutures between metatarsi are distinct on both the dorsal and plantar surfaces of proximal and distal parts of the shaft. Trochleae metatarsorum are largely cartilaginous in all individuals observed.

Adult—The shaft is relatively uniform in mediolateral width throughout the shaft. Extremitas proximalis tarsometatarsi is completely ossified with no trace of suture (Fig. 5E). Sulcus extensorius is clearly defined by ridges on the mediodorsal and laterodorsal margins of the proximal shaft. Tuberositas m. tibialis cranialis is a distinct pair of tubercles. Sutures between metatarsi are indiscernible. Trochleae metatarsorum are completely ossified.

Cerorhinca monocerata

Humerus (Fig. 6A)

Chick—The overall shaft is relatively strongly bowed in caudoventral view especially in young individuals (e.g., C1), and is approaching to the adult condition in older individuals (e.g., C8). Caput humeri and tuberculum ventrale are cartilaginous in all individuals (Fig. 6A). Crista m. supracoracoidei and linea m. latissimi dorsi are indiscernible in all individuals. Crista deltopectoralis is cartilaginous until ~10 days after hatching (C1–C3) and present as a thickened crest on the cranial margin of the proximal shaft in older individuals, but is not completely ossified in any case. Crista bicipitalis begins to ossify ~30 days old (C7). Fossa tricipitalis is not developed in the ossified part and the corresponding part is convex without crus dorsale fossae until ~35 days old (C1–C7); it is slightly concave with an indistinct crus dorsale fossae in an older individual (C8; Fig. 6A). A single foramen nutriens is present with a distinct opening on the ventral margin of shaft at the position one-third of bone length from the proximal end (Fig. 6A). Condyli dorsalis et ventralis and epicondylus dorsalis et ventralis are cartilaginous. Fossa brachialis is discernible only after ~30 days after hatching (C7, C8). Tuberculum supracondylare ventrale is indiscernible in any case. Fledgling—Morphology of the humerus is highly variable among fledglings; bones of some individuals are hardly more developed than those in old chicks, reflecting the variation of the timing to leave the nest and nutritional condition until it. Caput humeri is mostly cartilaginous in some individuals whereas it is ossified in others (Fig. 6A). Nevertheless, it is highly porous in all individuals examined. Tuberculum ventrale is cartilaginous in some individuals, but as well developed in others as in adults. Crista m. supracoracoidei is discernible but not as clearly marked as in adults. Linea m. latissimi dorsi is quite indistinct in most individuals. Fossa tricipitalis is slightly concave in most cases, but does not excavate caput humeri and tuberculum ventrale (Fig. 6A). Crus dorsale fossae is blunt at best. A single foramen nutriens is present on the ventral margin of shaft, with a prominent opening. Condyli dorsalis et ventralis are variably ossified, but always highly porous. In some individuals, both epicondylus are almost completely ossified with distinct muscular/ligamental pits, whereas in others they are only incompletely ossified. Fossa m. brachialis is present in all individuals examined, whose margins are variably distinct. Tuberculum supracondylare dorsale is completely unossified in some individuals whereas it is ossified and distinct in others.

Adult—Caput humeri is developed proximocaudally, bulbous, with the caudodistal margin being protruding distally and deeply excavated by fossa tricipitalis, and surrounded by few pores (Fig. 6A). Tuberculum ventrale is completely ossified and overhanging distally. Crista m. supracoracoidei is well marked and slightly elevated from the surrounding surface, distal to which linea m. latissimi dorsi is present. Fossa tricipitalis is well excavated and interrupted by sharp crus dorsale fossae. Foramen nutriens is lying on the ventral margin of the shaft distal to crista bicipitalis and with

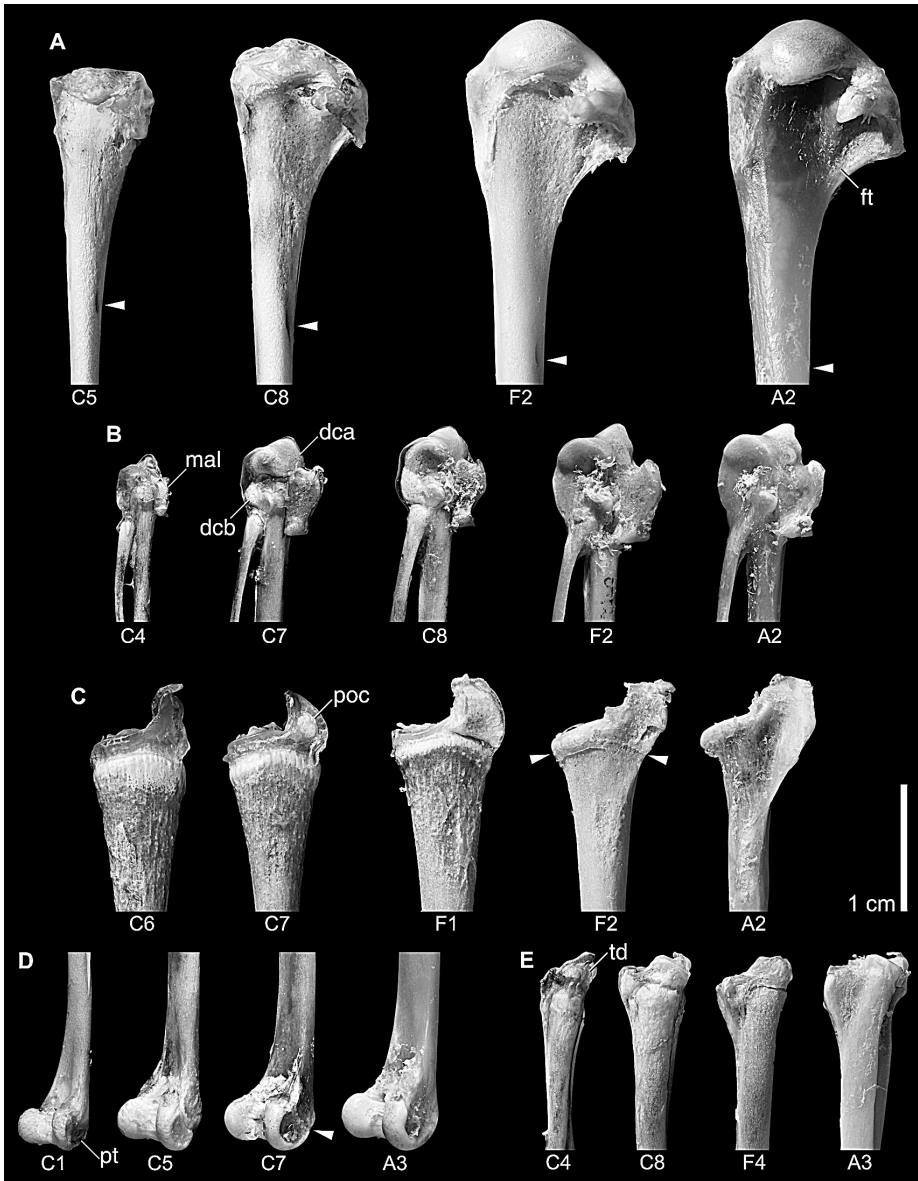


Fig. 6. Ontogenetic variation of macroscopic morphology of *Cerorhinca monocerata* limb bones. A, proximal end of humerus, caudoventral view, showing foramen nutriens (white arrowheads); B, proximal end of carpometaarpus, ventral view, showing the sequence of ossification of carpal and metacarpal elements; C, proximal end of tibiotarsus, medial view, showing the fusion of the proximal ossification center with a suture (white arrowheads in F2); D, distal end of tibiotarsus, craniolateral view, showing the fusion between the diaphysis and fused ossa proximalia tarsi with suture (white arrowhead in C7); E, proximal end of tarsometatarsus, medial view, showing the fusion between os tarsi distale and the fused shaft of metatarsi. Scale bar equals 1 cm. Labels below panels refer to individual codes in Table A4. Abbreviations: dca, the proximal os carpi distale; dcb, the distal os carpi distale; ft, fossa tricipitalis; mal, os metacarpale alulare; poc, proximal ossification center; pt, ossa proximalia tarsi; td, os tarsi distale.

a minute opening (Fig. 6A). Condyli dorsalis et ventralis are completely ossified and surrounded with few pores. Both epicondyli are completely ossified with prominent muscular/ligamental pits and protruding distally. Tuberculum supracondylare dorsale and fossa brachialis are relatively well marked compared to those in chicks and fledglings.

Ulna

Chick—In youngest chicks (e.g., C1), the midshaft is distinctly thinner than the proximal or distal parts, unlike older individuals. Extremitas proximalis ulnae, including olecranon, is almost completely cartilaginous. Tuberculum lig. collateralis ventralis and fossa m. brachialis are indistinct. Foramen nutiens is present on the cranial margin of the shaft one-third of the length down from the proximal end, with a distinct opening. Papillae remigales are not discernible as ossified structures. Linea intermuscularis on the cranial margin of the distal shaft is indiscernible. Extremitas distalis ulnae is cartilaginous but contains a few small ossifications in individuals older than ~10 days old (C3–C8).

Fledgling—As for the humerus, the degree of skeletal maturity of the ulna varies among individuals in this stage. The ossification of extremitas proximalis ulnae varies from a completely cartilaginous condition to almost completely developed cotylae, but the surface of the cotylae is generally porous. The tip of olecranon is cartilaginous in all cases. Tuberculum lig. collateralis ventralis is discernible as a blunt tubercle in most cases but its margins are indistinct. Margins of impressio m. brachialis are not well defined. Foramen nutiens is present on the cranial margin of the shaft with a distinct opening. At most, five papillae remigales are discernible on the caudal margin of the midshaft; in some individuals, papillae are completely indiscernible. Linea intermuscularis on the cranial margin of the distal shaft is, if discernible, indistinct. Extremitas distalis ulnae is almost as well ossified in some individuals as in adults, whereas almost completely cartilaginous in others.

Adult—Extremitas proximalis ulnae is ossified, and there are few pores on articular cotylae. Olecranon is completely ossified. Tuberculum lig. collateralis ventralis is distinct, triangular, and well elevated from the shaft. Impressio m. brachialis is marked by distinct margins. Foramen nutiens is present on the cranial margin of the shaft distal to impressio m. brachialis, with a minute opening. An array of at least 10 papillae remigales are discernible on the caudal margin of the shaft. Linea intermuscularis is distinct on the cranial margin of the distal half of the shaft. Extremitas distalis ulnae, including trochlea carpalis and tuberculum carpalis, is completely ossified.

Carpometacarpus (Fig. 6B)

Chick—Five distinct ossification centers are recognized as in *Calonectris leucomelas* and *Phalacrocorax capillatus*. Ossa metacarpale majus et minus are already ossified in the youngest individual (C1), os metacarpale alulare begins to ossify ~5 days after hatching (C2), the proximal carpus around ~25 days old (C6), and the distal carpus around ~30 days old (C7). They do not appear to fuse until around ~35 days old (C8), when only the two carpals are fused to each other (Fig. 6B). Trochlea carpalis is mostly cartilaginous, but the proximal margin is formed by the carpus from ~30 days old (C7 and older). Sulcus tendineus is indiscernible. Foramen nutiens is present on the caudal margin of the shaft of os metacarpale majus with a distinct opening. The distal articular surfaces are cartilaginous throughout the period.

Fledgling—Degree of ossification of the element varies among individuals; in some individuals all ossification centers are unfused, whereas in some others they are mostly fused (Fig. 6B). The suture between ossa metacarpalia alulare et majus is

distinct even in those individuals where all elements are fused. The suture between the proximal carpus and os metacarpale majus is discernible in some individuals (F1). On and around trochlea carpalis, minute pores are prevalent. Processus pisiformis is ossified in most cases. Sulcus tendineus is indistinct at best. Foramen nutrienis is present on the caudal margin of os metacarpale majus with a distinct but relatively small opening. The distal articular surfaces are mostly porous even when ossified.

Adult—All elements are completely fused with little trace of sutures (Fig. 6B). Trochlea carpalis is completely ossified and with few foramina. Sulcus tendineus is distinct on the dorsal margin of the distal three-fifth of the shaft. Foramen nutrienis is present on the caudal margin of os metacarpale majus, but with a quite minute opening. Distal articular surfaces are completely ossified with little porosity.

Femur

Chick—Both ends are at least partly cartilaginous through this stage. Caput femoris is little more than a medial extension of the proximal shaft in most individuals. Impressiones mm. et ligg. trochanteris are only discernible as indistinct depressions even in the oldest individual (~35 days old, C8), and indiscernible in younger ones. Linea intermuscularis cranialis and even crista trochanterica are indistinct. Foramen nutrienis is present at a variable position on the caudal or medial margin of the shaft with a relatively large opening. Tuberculum m. gastrocnemialis lateralis is present only in the oldest individual (C8) as an indistinct tubercle on the caudolateral margin of the distal shaft. Extremitas distalis femoris is cartilaginous, and in individuals over ~30 days old (C7, C8) contains an unfused ossification center on the distal end of condylus lateralis.

Fledgling—Degree of ossification varies among individuals in this stage. Caput femoris is at least partly ossified, but the margins are not as distinct as in adults. In all individuals, the surfaces of caput femoris and facies articularis antitrochanterica are porous. In some individuals, impressiones mm. et ligg. trochanteris are present as depressions on the lateral surface of the proximal shaft, but their margins are not well delineated. Linea intermuscularis cranialis is indistinct and does not reach the midpoint as in adults. Foramen nutrienis is present at a variable position on the caudal margin of the shaft, and its opening is small but more distinct than that in adults. The development of tuberculum m. gastrocnemialis lateralis is variable among individuals; it is almost absent in some individuals, whereas as well developed as in adults in others. In some individuals, extremitas distalis femoris is ossified but porous, whereas in others it is cartilaginous and contains an ossification center on the distal end of condylus lateralis. The ossification center appears to extend medially before fusing to the diaphysis, and a suture is observable in most individuals in this stage.

Adult—Both ends are ossified. Caput femoris is bulbous, well delineated by distinct margins, and with few pores on its surface. Impressiones mm. et ligg. trochanteris are well developed as pits on the surface lateral to trochanter femoris. Linea intermuscularis cranialis extends from the distal end of crista trochanterica and over the midpoint. Foramen nutrienis is present at a variable position on the caudal or caudomedial margin of the shaft, with a very minute opening. Tuberculum m. gastrocnemialis lateralis is present as rugosity on the caudolateral margin of the shaft just proximal to condylus lateralis. Extremitas distalis femoris is completely ossified with few pores on the articular condyles.

Tibiotarsus (Fig. 6C, D)

Chick—The proximal end is disproportionately thick craniocaudally and the distal end is wide mediolaterally in youngest chicks. Extremitas proximalis tibiotarsi is

cartilaginous throughout this stage, and in individuals over ~30 days old (C7, C8), an ossification center is present in the cranial portion of proximal cartilage within crista cnemialis cranialis (Fig. 6C). Cristae cnemiales cranialis et lateralis are almost entirely cartilaginous and do not form a sharp ridge. Cranial and caudal margins of the proximal shaft are convex rather than concave. Crista fibularis is indistinct in chicks under ~35 days old (C1–C7), whereas it is a blunt ridge on the lateral margin of the shaft in the oldest chick (C8). Foramen nutrienis is present on the lateral margin of the shaft caudal to fibula, and its opening is relatively large, forming a small fossa in young chicks (up to ~25 days old, C1–C4). Extremitas distalis tibiotarsi is cartilaginous in young chicks, within which the fused ossa proximalia tarsi are observed (Fig. 6D). Ossa proximalia tarsi first form the cranial part of distal condyles (C1–C6), extend caudally to cover the entire distal end (~30 days old, C7), and then fuse with the diaphysis with a distinct suture (~35 days old, C8).

Fledgling—The overall profile is similar to that in adults. Extremitas proximalis tibiotarsi is either cartilaginous with a distinct ossification center or ossified and fusing with the proximal epiphysis with a prominent suture (Fig. 6C). Cristae cnemiales cranialis et lateralis are mainly formed by the proximal ossification center, and are blunt and short longitudinally. Crista fibularis is indistinct in some individuals whereas it is as well developed as in adults. Foramen nutrienis has a relatively large and longitudinally long opening. The degree of ossification in extremitas distalis tibiotarsi and fusion between the diaphysis and the fused ossa proximalia tarsi are variable, from complete separation to an almost completely fused condition (Fig. 6D), but the articular surface of the condyles, especially the caudodistal part, is rather porous. Pons supratendineus is a cartilaginous bridge formed between the diaphysis and ossa proximalia tarsi.

Adult—The shaft is relatively straight and almost uniform in width throughout the length. Extremitas proximalis tibiotarsi is ossified with no trace of suture (Fig. 6C). The cranial surface of the proximal shaft, facies gastrocnemialis, and fossa flexoria are concave and the shaft is strongly tapered at this position. Crista cnemialis cranialis is thin and sharp, triangular in medial view, and extending as distal as the position of the proximal end of impressio lig. collateralis medialis. Crista fibularis is developed as a thin ridge. Foramen nutrienis is present on the lateral margin of the shaft between the distal end of crista fibularis and the distal junction with the fibula, with a minute opening. Extremitas distalis tibiotarsi is ossified with no trace of suture (Fig. 6D) and there are few pores on the articular surface of the distal condyles. Pons supratendineus is present as an ossified bridge over sulcus extensorius.

Tarsometatarsus (Fig. 6E)

Chick—Extremitas proximalis tarsometatarsi is cartilaginous and contains os tarsi distale as a single ossification center. The ossification of os tarsi distale forms only the proximal articular surface in young chicks, but then extends caudally to form hypotarsus in older chicks (~20 days and older, C5–C8; Fig. 6E). Sulcus extensorius is broad and ill-defined. Sutures between metatarsi are distinct on both the dorsal and plantar surfaces throughout the shaft until ~20 days old, and become indistinct thereafter. Tuberositas m. tibialis cranialis is indiscernible. Foramen vasculare distale is proximodistally long. Trochleae metatarsorum are at first cartilaginous and then gradually become ossified. Even in the oldest individual (~35 days old, C8), margins of the distal trochleae are not completely defined. The surface of the trochleae is rather porous.

Fledgling—Degree of ossification is variable among individuals. Os distale tarsi is at least forming the proximal articular surface and the proximal end of hypotarsus, and in most individuals, it is fusing to the diaphysis of fused metatarsi. The fusion

appears to begin in the hypotarsus and then below the proximal articular surface (Fig. 6E). Margins of sulcus extensorius are blunt. Sutures between metatarsi, especially the one between metatarsi II and III, are still discernible (although indistinct) on both the dorsal and plantar surfaces. Tuberositas m. tibialis cranialis is indiscernible. Foramen vasculare distale is relatively long proximodistally. Trochleae metatarsorum in some individuals are nearly as well ossified as in adults. Surfaces of the distal trochleae are not distinctively porous.

Adult—Extremitas proximalis tarsometatarsi is completely ossified with little trace of suture (Fig. 6E). Margins of sulcus extensorius are well defined, and the lateral one extends on the dorsolateral margin of the shaft over the midpoint. Sutures between metatarsi are indiscernible. Tuberositas m. tibialis cranialis is present in the proximal part of sulcus extensorius as paired tubercles. Foramen vasculare distale is relatively shorter than in chicks and fledgelings, probably because of the complete fusion between metatarsi. Few pores are present on the surface of distal trochetae.

Morphometrics

As measures of longitudinal and circumferential growth of bones, respectively, length and shaft width (dorsoventral for wing elements and mediolateral for leg elements) of six limb bones were recorded, which are shown in Figs 7, 8 (for raw data, see Appendix B and Watanabe & Matsuoka, 2013). Disregarding the potential confounding factor of sexual dimorphism due to small sample sizes, it was observed that lengths of limb bones reach adult size range of each species during the chick period, perhaps except for *Cerorhinca monocerata* (sampling of chicks for *Larus crassirostris* was too incomplete to span the full variation within the period; Figs 7A, C, E, 8A, C). In contrast, the shaft width tends not to reach the adult size range during the chick period and to be still smaller in fledglings and juveniles than in adults (Figs 7B, D, F, 8B, D). This tendency is especially clear for the humerus and ulna of *Ardea cinerea* and most elements in *Calonectris leucomelas* and *Cerorhinca monocerata*. In *Ardea cinerea*, *Phalacrocorax capillatus*, and *Larus crassirostris*, shaft width of tarsometatarsus was distinctively larger in some immature individuals than in adults because the width was measured on the midshaft region which is swollen in immature individuals (see above).

The Kruskal-Wallis test was conducted to confirm the possible difference of length and shaft width of limb bones between fledglings and adults in *Calonectris leucomelas* (sample sizes were too small for the other species for testing). After controlling the false discovery rate (Benjamini & Hochberg, 1995) of 12 multiple hypothesis tests, significant differences were found in shaft widths of the humerus, ulna, carpometacarpus, and tarsometatarsus (Table 1). The results indicate that the longitudinal growth of long bones after the chick period is of negligible amount, whereas the circumferential growth of some elements is considerable in this species.

DISCUSSION

Ontogeny of long bones

Long bones grow both longitudinally and circumferentially. Longitudinal growth occurs through endochondral ossification in epiphyseal growth plates (e.g., Wolbach & Hegsted, 1952; Starck, 1996), whereas circumferential growth occurs through membranous ossification in the periosteum (e.g., Castanet *et al.*, 1996, 2000). In the ontogenetic series of the five species observed in this study and in Watanabe & Matsuoka (2013), most long bones of small chicks have cartilaginous epiphyses, whereas those of chicks and fledglings that are as large as adults have ossified epiphyses, consistently with the general pattern of longitudinal growth at epiphyseal

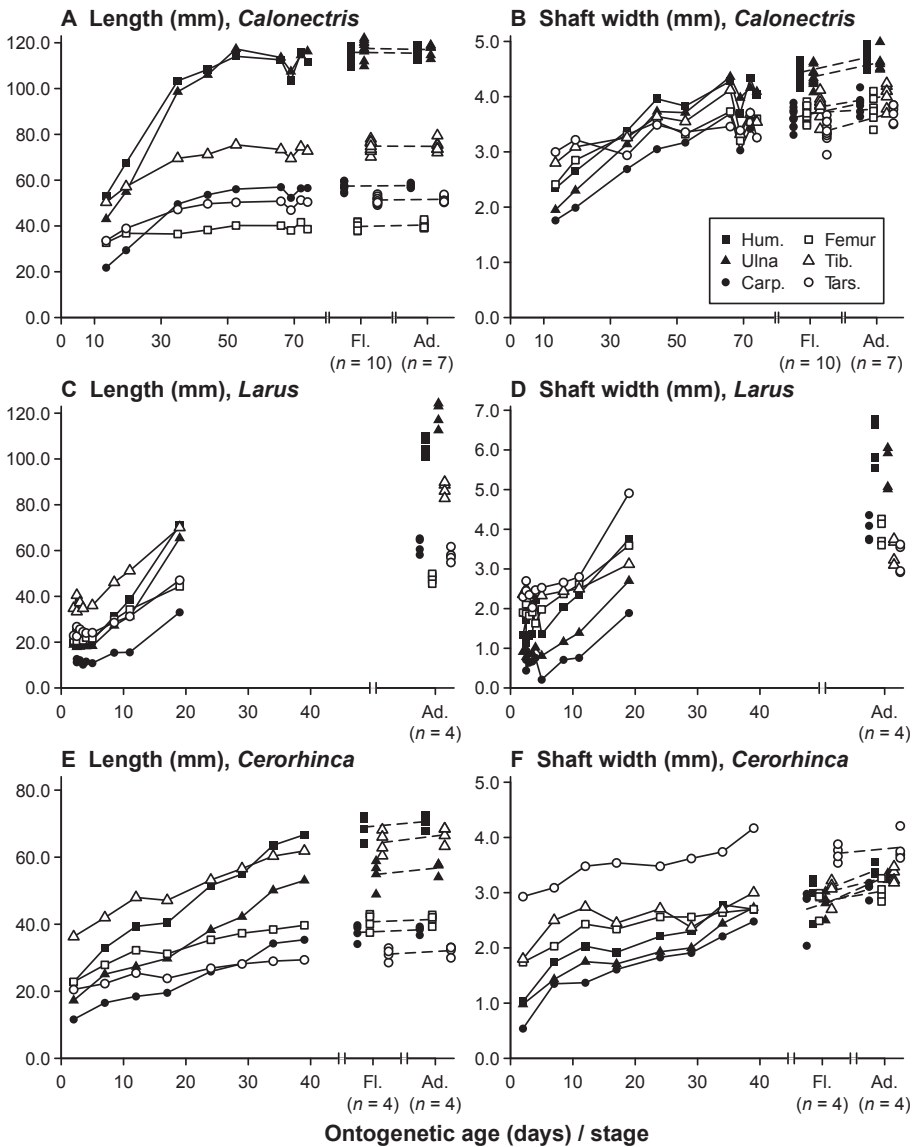


Fig. 7. Ontogenetic variation long bone length (A, C, F) and shaft width (B, D, F) of *A. B. Calonectris leucomelas*; C, D, *Larus crassirostris*; and E, F, *Cerorhinca monocerata*. For graphical clarity, points for fledglings (Fl.) and adults (Ad.) are horizontally displaced; points for chicks of adjacent ages are connected by solid lines, and mean values of fledglings and adults are connected by broken lines. Legend: solid square, humerus; solid triangle, ulna; solid circle, carpometacarpus; open square, femur; open triangle, tibiotarsus; open circle, tarsometatarsus.

growth plates. In general, the longitudinal growth seems to cease around the time of fledging, when lengths of limb bones are as large as those in adults (Figs 7, 8) and little trace of epiphyseal growth plates remains with a few exceptions (Figs 1–4, 6). In *Calonectris leucomelas* where the nesting period is prolonged as in most

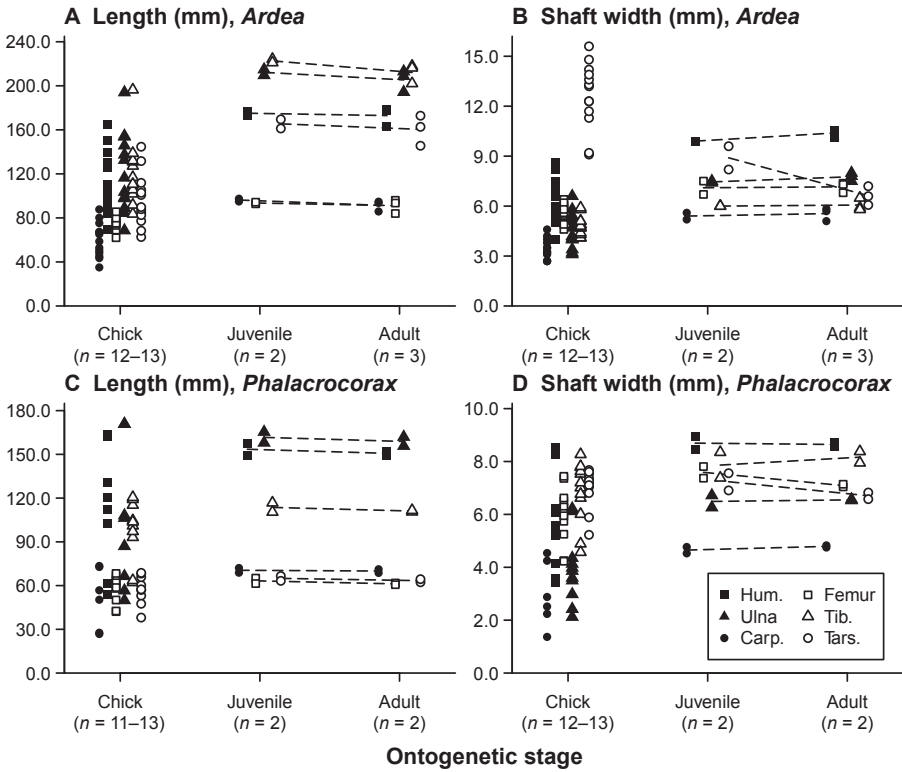


Fig. 8. Ontogenetic variation of long bone length (A, C) and shaft width (B, D) in: A,B, *Ardea cinerea*; and C,D, *Phalacrocorax capillatus*. For graphical clarity, points are horizontally displaced, and mean values of juveniles and adults are connected by broken lines. For graphical clarity, points for fledglings (Fl.) and adults (Ad.) are horizontally displaced; points for chicks of adjacent ages are connected by solid lines, and mean values of fledglingjuveniles and adults are connected by broken lines. Legend: solid square, humerus; solid triangle, ulna; solid circle, carpometacarpus; open square, femur; open triangle, tibiotarsus; open circle, tarsometatarsus.

procariiform birds (Carboneras, 1992), long bones reach the adult size ranges well before the fledging. In *Cerorhinca monocerata* where chicks are not fully grown at the time of fledging as in other alcid (Gaston & Jones, 1998), largest chicks and some fledglings have limb bones that are not fully grown and with cartilaginous epiphyses. As originally mentioned by Watanabe & Matsuoka (2013), traces of epiphyseal growth plates are observed in the proximal ends of tibiotarsus and tarsometatarsus in juveniles of *Ardea cinerea* where the longitudinal growth appears to be prolonged.

Circumferential growth of long bones, in contrast, does not appear to cease at the same time in most species observed. In most cases, shaft widths of limb bones tend to be smaller in chicks and fledglings/juveniles than in adults, except in tarsometatarsus in some species where thinning of the shaft occurs in late development. Although the sample size is sufficient for statistical tests only in *Calonectris leucomelas* (Table 1), visual inspection of Figs 7, 8 suggests this pattern is common in most other species examined, implying that circumferential growth of avian long bones generally continues for a certain period after the cessation of longitudinal growth. This result is consistent with the observation in *Passer domesticus* that most long

Table 1. Summary of individual data on ontogenetic series of *Calonectris leucomelas*. All individuals are from Awashima Island. Absolute ages (in days old, d.o.) are shown for chicks where available. Exact ages are not known for those simply labeled as “Chick”. Chicks are ordered primarily by age and then roughly by body size. Collection dates (Coll. date) are shown in the order of year, month, and day. Dash (—) denotes unknown for that individual. Abbreviations: BM, body mass (g); WL, wing (chord) length (mm); TS, tarsus length (mm).

Number	Age/Stage	Sex	Coll. date	BM	WL	TS
CL-C9	Chick	—	2013.08.27	192	39	32.2
CL-C1	13–14 d.o.	—	2013.08.30	228	42	35.7
CL-C2	19–20 d.o.	—	2013.08.30	335	54	41.1
CL-C3	33–37 d.o.	—	2013.09.21	532	142	49.6
CL-C4	42–46 d.o.	—	2013.09.25	630	162	50.7
CL-C5	51–54 d.o.	—	2013.10.05	600	200	52.0
CL-C6	64–68 d.o.	—	2013.10.15	680	270	52.2
CL-C8	Chick	—	2013.10.05	603	210	53.4
CL-C10	69 d.o.	—	2014.10.28	494	186	48.9
CL-C7	68–76 d.o.	—	2013.10.21	503	272	52.4
CL-C11	74 d.o.	—	2014.11.04	571	251	51.4
CL-F1	Fledgling	—	2012	c. 552	322	54.7
CL-F2	Fledgling	—	2012	c. 524	315	53.6
CL-F3	Fledgling	—	2012	c. 448	312	50.4
CL-F4	Fledgling	—	2014.11.18	528	312	53.9
CL-F5	Fledgling	—	2014.11.17	503	308	51.2
CL-F6	Fledgling	—	2014.11.15	624	322	53.3
CL-F7	Fledgling	—	2014.11.18	517	311	52.9
CL-F8	Fledgling	—	2014.11.14	493	317	50.4
CL-F9	Fledgling	—	2014.11.14	533	312	52.2
CL-F10	Fledgling	—	2014.11.14	440	304	51.3
CL-A1	Adult	Male	2013.09.25	583	317	52.2
CL-A2	Adult	Female	2013.10.04	492	320	51.8
CL-A3	Adult	Male	2013.10.06	676	327	54.9
CL-A4	Adult	Male	2013.10.14	599	313	51.9
CL-A5	Adult	Female	2013.10.17	497	313	51.5
CL-A6	Adult	Male	2013.10.12	557	317	54.0
CL-A7	Adult	Male	2013.10.09	572	321	52.4

bones of adults are significantly thicker, but not longer, than those of first year birds in females (although not so in males; Bjordal, 1987).

The proximal shafts of tibiotarsus and tarsometatarsus tend to be considerably thicker in large chicks than in adults (Figs 2C, 4A, D, 5C, E, 6C, E; see also Watanabe & Matsuoka, 2013: fig. 8B, D). According to Shufeldt (1886), this was a well known condition for tibiotarsus. One possible explanation for the thickness is that the thick cortex in leg bones of chicks compensates for weak immature bone tissue, providing the bones with sufficient strength to sustain growing body weight, as suggested for

Table 2. Summary of individual data on ontogenetic series of *Phalacrocorax capillatus*. All individuals are from Teuri Island, except for J1 and J2 which are from Haboro, Japan. See Table 1 for legend.

Number	Age/Stage	Sex	Coll. date	BM	WL	TS
PH-C11	Chick	—	2013.07.02	> 83	—	c. 33.8
PH-C12	Chick	—	2013.07.27	—	—	—
PH-C13	Chick	—	2013.06.11	224	50	40.4
PH-C10	Chick	—	2013.06.15	—	73	49.1
PH-C7	Chick	—	2014.07.02	—	—	53.8
PH-C2	Chick	—	2013.07.27	—	112	57.4
PH-C1	Chick	—	2013.07.27	—	94	50.5
PH-C14	Chick	—	2013.06.16	—	113	59.4
PH-C6	Chick	—	2014.07.02	—	—	59.6
PH-C8	Chick	—	2014.07.02	—	—	—
PH-C3	Chick	—	2013.07.27	—	150	c. 65.0
PH-C5	Chick	—	2014.07.02	—	—	67.2
PH-C4	Chick	—	2013.07.27	—	225	70.8
PH-C9	Chick	—	2013.07.14	1332	264	70.3
PH-C15	Chick	—	2013.07.27	—	283	69.8
PH-J1	Juvenile	Male?	2013.08.01	1474	321	69.1
PH-J2	Juvenile	Female?	2015.07.23	1055	305	64.7
PH-A1	Adult	Female	2013.05.26	2650	317	64.2
PH-A2	Adult	Female	2014.06	2574	313	67.7

Ardea cinerea by Watanabe & Matsuoka (2013) (see also Carrier & Leon, 1990).

Although longitudinal growth of limb bones mostly ceases around the time of fledging, ossified epiphyses in large chicks and juveniles tend to be characterized by higher porosity of articular surfaces and more frequent occurrence of minute foramina surrounding them than those in adults (e.g., Fig. 3A). Apart from the ossification of epiphyseal cartilages, some macroscopic osteological landmarks show prominent morphological changes through the postnatal ontogeny. In general, most prominent osteological landmarks in adults, such as crista deltopectoralis of humerus and cristae cnemiales cranialis et lateralis of tibiotarsus, are mostly cartilaginous and not prominent on immature bones of chicks. Attachment sites for muscles and ligaments are also indiscernible in chick bones. Most of these structures develop in the chick stage and become discernible in limb bones of large chicks and fledglings/juveniles.

Foramen nutriens shows conspicuous ontogenetic variation, both in size and number within a bone. It tends to have a larger opening in chicks and juveniles than in adults (Figs 1B, C, 2A, 3B–D, 5A, 6A). In addition to large size, more than one foramen nutriens may be present on the humerus and ulna of chicks in *Phalacrocorax capillatus*, as well as in *Ardea cinerea* (see Watanabe & Matsuoka, 2013), where only one is observed in adults (Fig. 3B, C). The tarsometatarsus of *Calonectris leucomelas* and the carpometacarpus, femur, and tibiotarsus of *Phalacrocorax capillatus* also may have additional foramina nutrientia during the chick stage. Diameter of foramen nutriens is considered to be related to the amount of blood supply to the

Table 3. Summary of individual data on ontogenetic series of *Larus crassirostris*. All individuals are from Rishiri Island. See Table 1 for legend.

Number	Age/Stage	Sex	Coll. date	BM	WL	TS
LA-C5	1–3 d.o.	—	2014.06.23	29.0	21	23.3
LA-C4	1–4 d.o.	—	2014.06.15	44.9	22	23.3
LA-C6	2–5 d.o.	—	2014.06.20	29.6	23	23.9
LA-C2	4 d.o.	—	2014.06.15	32.6	21	24.5
LA-C7	4–6 d.o.	—	2014.06.27	32.8	22	24.1
LA-C8	3 d.o.	—	2014.06.15	47.3	—	—
LA-C9	1–4 d.o.	—	2014.06.20	42.6	24	26.0
LA-C10	7–10 d.o.	—	2014.06.23	55.4	28	28.3
LA-C3	11 d.o.	—	2014.06.23	31.0	31	31.1
LA-C1	19 d.o.	—	2014.06.28	296	84	46.3
LA-A1	Adult	Male	2014.06.19	581	377	57.1
LA-A2	Adult	Male	2014.06.19	680	378	59.7
LA-A3	Adult	Female	2014.06.19	506	350	55.0
LA-A4	Adult	Female	2014.06.24	437	387	53.6

Table 4. Summary of individual data on ontogenetic series of *Cerorhinca monocerata*. All individuals are from Teuri Island. See Table 1 for legend.

Number	Age/Stage	Sex	Coll. date	BM	WL	TS
CM-C1	0–4 d.o.	—	2013.06.24	52.7	22	20.8
CM-C2	5–9 d.o.	—	2013.06.24	141	32	23.2
CM-C3	10–14 d.o.	—	2013.06.24	174	41	26.8
CM-C4	15–19 d.o.	—	2013.06.24	197	43	25.0
CM-C5	22–26 d.o.	—	2013.07.16	180	58	27.8
CM-C6	27–31 d.o.	—	2013.07.16	210	77	28.7
CM-C7	32–36 d.o.	—	2013.07.16	243	107	29.7
CM-C8	37–41 d.o.	—	2013.07.16	285	112	30.7
CM-F1	Fledgling	—	2013.07.16	305	145	33.0
CM-F2	Fledgling	—	2013.07.24	360	155	31.6
CM-F3	Fledgling	—	2013.07.30	> 73	115	29.7
CM-F4	Fledgling	—	2013.07.30	308	151	30.7
CM-A1	Adult	Male	2014.07.03	—	179	31.6
CM-A2	Adult	Female	2014.07.13	> 488	176	29.8
CM-A3	Adult	Male	2014.05.26	545	185	32.3
CM-A4	Adult	Male	2013.07.06	610	195	31.6

bone (Seymour *et al.*, 2012 and references therein). It is reasonable to consider that large diameters of (multiple) foramina nutrientia are associated with an active bone metabolism during the skeletal development. At the same time, the presence of such

Table 5. Length (L) and shaft width (SW) of limb bones in *Calonectris leucomelas* (mm).

Number	Humerus		Ulna		Carp.		Femur		Tibiotarsus		Tars.	
	L	SW	L	SW	L	SW	L	SW	L	SW	L	SW
CL-C9	48.6	2.5	37.9	4.8	20.3	1.4	29.2	2.6	45.5	2.7	30.5	3.0
CL-C1	53.3	2.3	43.0	2.0	21.8	1.8	32.7	2.4	50.4	2.8	33.6	3.0
CL-C2	67.5	2.7	54.9	2.3	29.4	2.0	36.8	2.9	57.2	3.1	39.0	3.2
CL-C3	103.4	3.4	98.6	3.1	49.5	2.7	36.5	3.3	69.5	3.3	47.2	2.9
CL-C4	108.3	4.0	106.0	3.7	53.6	3.1	38.3	3.5	71.2	3.6	49.7	3.5
CL-C5	114.1	3.8	117.3	3.7	56.1	3.2	40.2	3.3	75.4	3.6	50.4	3.4
CL-C6	112.5	4.3	113.6	4.4	57.0	3.7	40.2	3.7	73.4	4.1	50.8	3.5
CL-C8	116.1	4.4	118.5	4.2	58.9	3.3	41.4	3.8	76.2	3.7	52.1	3.4
CL-C10	103.6	3.7	107.5	4.0	52.3	3.0	38.1	3.2	69.5	3.3	47.0	3.4
CL-C7	115.5	4.3	114.6	4.2	56.5	3.4	41.6	3.5	74.6	3.7	51.3	3.7
CL-C11	111.7	4.0	116.3	4.1	56.6	3.3	38.6	3.6	72.8	3.5	50.5	3.3
CL-F1	118.5	4.4	120.0	4.3	59.5	3.6	41.3	3.8	78.1	3.9	53.9	3.3
CL-F2	118.4	4.5	119.1	4.4	59.5	3.8	40.6	3.9	76.5	4.0	52.1	3.3
CL-F3	109.7	4.5	109.8	4.3	54.3	3.6	37.8	3.7	70.2	3.6	49.1	3.6
CL-F4	118.5	4.7	121.3	4.6	59.8	3.9	41.9	3.8	76.7	4.1	53.0	3.7
CL-F5	113.6	4.6	116.2	4.6	57.7	3.7	38.6	3.7	73.4	3.8	50.4	3.5
CL-F6	117.3	4.5	120.3	4.5	57.0	3.8	39.8	3.8	76.3	3.8	51.9	3.3
CL-F7	117.8	4.3	122.0	4.3	57.7	3.6	41.7	3.6	76.6	3.9	51.9	3.3
CL-F8	113.0	4.4	111.9	4.3	54.9	3.3	37.9	3.7	72.7	3.9	49.7	3.4
CL-F9	116.9	4.3	117.6	4.2	57.1	3.5	40.2	3.5	73.6	3.8	51.0	3.6
CL-F10	114.7	4.2	117.1	4.1	56.5	3.5	38.0	3.5	74.8	3.4	50.3	3.0
CL-A1	112.3	4.5	112.9	4.5	56.6	3.8	39.1	3.8	72.1	3.8	50.8	3.7
CL-A2	115.3	4.6	118.8	4.5	57.8	3.6	39.2	3.4	73.8	3.7	50.3	3.5
CL-A3	118.8	4.9	119.0	5.0	58.6	4.2	42.8	3.7	79.5	4.2	53.8	3.9
CL-A4	114.9	4.8	117.9	4.6	57.7	3.9	39.4	4.0	73.5	4.1	51.3	3.9
CL-A5	115.9	4.6	118.0	4.5	57.1	3.7	39.4	3.6	73.6	4.0	50.6	3.5
CL-A6	117.0	4.9	118.1	4.6	58.9	3.9	41.9	4.1	75.7	4.2	53.0	3.8
CL-A7	114.1	4.6	114.6	4.6	56.8	3.9	41.0	3.8	75.3	4.1	51.7	3.8

an ontogenetic variation necessitates controls of ontogenetic stages in comparative analyses depending on the diameter (Seymour *et al.*, 2012; Allan *et al.*, 2014).

Ossification centers

Although the presence of epiphyseal ossification centers in long bones of birds has not been widely accepted (Baumel & Witmer, 1993; but see also Starck, 1994), the one in the proximal tibiotarsus is considered exceptional (e.g., Haines, 1942) and has been described in various taxa: “Gallinaceae and Ratitae” (Parsons, 1905), *Rhea americana* (greater rhea; Fuchs, 1908), *Apteryx mantelli* (northern brown kiwi; Beale, 1985, 1991), *Dinornis* (giant moa; Turvey & Holdaway, 2005), *Gallus* (domestic fowl; Church & Johnson, 1964; Hogg, 1980), *Centrocercus urophasianus* (sage grouse; Shufeldt, 1881), *Pelecanoides urinatrix exsul* (common diving petrel; Barnett & Lewis, 1958), *Grus grus* (common crane; Serjeantson, 1998), several otidid species (Naldo *et al.*, 1997, 1998), *Ardea cinerea* (gray heron; Watanabe & Matsuoka, 2013), *Geococcyx californianus* (greater roadrunner; Shufeldt, 1886), and *Troglodytes aedon* (house wren; Huggins *et al.*, 1942). Although this ossification center is sometimes referred to as “patella” in the literature, it is a distinct structure from the true patella (Livezey & Zusi, 2006).

The presence of an epiphyseal ossification center in the proximal tibiotarsus was confirmed in *Calonectris leucomelas*, *Phalacrocorax capillatus*, and *Cerorhinca monocerata* (Figs 2C, 4A, 6C), although sampling was too poor in *Larus crassirostris*

Table 6. Length (L) and shaft width (SW) of limb bones in *Phalacrocorax capillatus* (mm).

Number	Humerus		Ulna		Carp.		Femur		Tibiotarsus		Tars.	
	L	SW	L	SW	L	SW	L	SW	L	SW	L	SW
PH-C11	c. 53.6	3.6	49.9	2.1	27.7	1.4	42.1	4.3	62.2	4.9	36.4	5.6
PH-C13	61.6	3.4	56.6	2.4	26.9	2.2	42.6	4.2	63.6	4.6	38.1	5.9
PH-C10	—	4.2	c. 66.4	3.0	34.3	1.8	50.1	5.3	—	6.0	47.6	7.1
PH-C7	—	—	c. 95.1	4.2	—	—	63.5	6.0	99.2	6.5	—	7.3
PH-C2	c. 89.3	5.4	c. 86.7	3.9	—	—	63.1	6.3	c. 101.	17.5	56.9	7.6
PH-C1	c. 102.4	5.2	c. 87.0	3.5	c. 40.8	3.2	58.1	5.7	93.1	7.8	53.1	7.2
PH-C14	112.5	5.2	106.4	3.9	50.3	2.5	58.6	6.0	97.2	6.8	57.5	7.6
PH-C6	—	—	—	—	—	—	62.2	6.3	103.7	6.6	57.0	7.4
PH-C8	120.3	5.6	108.1	3.6	51.3	3.4	—	—	—	—	—	—
PH-C3	c. 130.5	6.1	129.7	4.1	56.8	2.9	58.8	6.3	104.2	7.9	60.1	7.4
PH-C5	145.7	6.5	144.2	4.5	c. 64.3	3.2	64.2	6.6	115.2	7.0	65.8	7.5
PH-C4	159.3	7.9	166.3	5.7	73.7	4.1	58.8	6.5	104.6	7.6	60.3	7.7
PH-C9	162.4	8.3	170.8	6.2	73.5	4.3	66.8	7.4	119.2	8.3	68.3	7.0
PH-C15	163.5	8.5	170.8	6.1	72.8	4.5	68.3	7.4	120.5	7.8	68.8	6.8
PH-J1	157.6	8.9	165.4	6.7	72.1	4.8	65.1	7.8	116.8	8.4	66.6	7.6
PH-J2	149.4	8.5	157.9	6.3	68.9	4.5	61.4	7.4	110.4	7.4	63.3	6.9
PH-A1	149.5	8.7	155.8	6.6	68.7	4.8	61.8	7.0	110.6	8.4	62.3	6.8
PH-A2	152.0	8.6	161.9	6.5	71.2	4.8	60.6	7.1	111.6	8.0	64.4	6.6

to confirm the presence of the ossification center in this species. There seems to be interspecific variation in the position of the appearance of the ossification center within the proximal epiphysis of tibia; it first appears in the cranial margin of the epiphysis in the three species examined here, whereas it appears in a more caudal position in *Ardea cinerea* (see Watanabe & Matsuoka, 2013). Its fusion with diaphysis apparently coincides with the cessation of longitudinal growth of the bone before or around the fledging, and the suture disappears before the adult stage. The prevalence of this structure across various modern birds including Palaeognathae, Galloanserae and Neoaves suggests that the presence of this ossification center is a primitive condition within Neornithes, but it is not clear if it was present in more basal birds.

Watanabe & Matsuoka (2013) reported the presence of ossification centers in the proximal and distal epiphyses of the femur in *Ardea cinerea*. The presence of the latter was confirmed in *Phalacrocorax capillatus* and *Cerorhinca monocerata*, whereas this was not observed in *Calonectris leucomelas*. The former was not observed in the species examined in this study. Another, rather indistinct, ossification center was observed in the distal epiphysis of ulna in *Cerorhinca monocerata*. The process of fusion of these ossification centers could not be observed in detail.

Interestingly, crista fibularis of tibiotarsus is formed by a distinct ossification center in *Phalacrocorax capillatus*; the ossification center is present in large chicks and fused to the diaphysis of tibia in largest chicks with a prominent suture (Fig. 4B). In *Gallus*, the crest originates from a distinct cartilaginous condensation in the embryonic development, but ossifies through the extension of the tibial diaphysis in the postnatal stage (Müller & Streicher, 1989). The apparent absence of this ossification center in the other species examined in this study, as well as phylogenetic relationships among them, suggests that the condition in *Phalacrocorax capillatus* is a derived condition. In the proximal tarsometatarsus of *Calonectris leucomelas*, a separate ossification center for arcus extensorius was observed to appear within retinaculum extensorium tarsometatarsi during the juvenile stage, which then fuses

Table 7. Length (L) and shaft width (SW) of limb bones in *Larus crassirostris* (mm).

Number	Humerus		Ulna		Carp.		Femur		Tibiotarsus		Tars.	
	L	SW	L	SW	L	SW	L	SW	L	SW	L	SW
LA-C5	19.8	1.3	18.9	0.9	—	—	20.6	1.9	34.8	2.4	23.0	2.3
LA-C4	18.9	1.1	17.9	0.8	11.2	0.4	20.5	1.9	33.3	2.3	22.6	2.5
LA-C6	19.8	1.4	19.1	0.9	10.2	0.7	20.7	1.9	34.8	2.1	24.6	2
LA-C2	21.4	1.2	18.2	1.0	11.5	0.8	22.0	1.6	36.9	2.3	24.0	2.5
LA-C7	20.9	1.4	18.4	0.8	10.9	0.2	21.7	2.0	36.0	2.3	24.2	2.5
LA-C8	23.0	1.3	18.0	0.9	12.2	0.6	23.3	1.8	37.3	2.3	25.7	2.4
LA-C9	24.2	1.7	21.4	1.0	12.7	0.7	24.7	2.1	40.5	2.5	26.8	2.7
LA-C10	31.2	2.0	27.2	1.2	15.4	0.7	29.9	2.4	46.1	2.4	28.6	2.7
LA-C3	38.5	2.4	31.3	1.4	15.6	0.8	34.2	2.6	51.2	2.5	31.3	2.8
LA-C1	71.2	3.8	65.4	2.7	33.1	1.9	44.3	3.6	70.1	3.1	47.1	4.9
LA-A1	109.8	6.8	123.0	5.9	64.7	4.1	49.0	4.3	88.7	3.7	58.1	3.6
LA-A2	108.1	6.6	124.3	6.1	65.4	4.4	49.8	4.1	89.9	3.8	61.7	3.6
LA-A3	104.0	5.8	117.0	5.1	60.6	3.8	47.2	3.7	86.0	3.2	56.9	2.9
LA-A4	100.7	5.6	112.6	5.0	58.2	3.7	45.5	3.6	82.8	3.1	54.8	3.0

Table 8. Length (L) and shaft width (SW) of limb bones in *Cerorhinca monocerata* (mm).

Number	Humerus		Ulna		Carp.		Femur		Tibiotarsus		Tars.	
	L	SW	L	SW	L	SW	L	SW	L	SW	L	SW
CM-C1	22.8	1.0	17.3	1.0	11.6	0.5	22.8	1.7	36.3	1.8	20.5	2.9
CM-C2	32.9	1.7	25.1	1.4	16.6	1.4	27.9	2.0	42.0	2.5	22.3	3.1
CM-C3	39.4	2.0	27.4	1.8	18.5	1.4	32.3	2.4	48.0	2.7	25.5	3.5
CM-C4	40.4	1.9	29.8	1.7	19.6	1.6	31.2	2.3	47.1	2.5	23.9	3.5
CM-C5	51.6	2.2	38.3	1.9	25.9	1.8	35.4	2.6	53.2	2.7	26.9	3.5
CM-C6	54.9	2.3	42.2	2.0	28.2	1.9	37.3	2.6	56.6	2.4	28.2	3.6
CM-C7	63.6	2.8	50.1	2.4	34.3	2.2	38.4	2.6	60.3	2.7	29.0	3.7
CM-C8	66.7	2.7	53.1	2.7	35.4	2.5	39.7	2.7	61.9	3.0	29.4	4.2
CM-F1	71.3	3.2	56.8	2.9	39.0	3.0	43.0	2.9	68.2	3.1	32.9	3.9
CM-F2	72.2	3.0	58.8	2.8	39.7	2.9	42.3	2.9	66.0	3.1	31.9	3.7
CM-F3	64.0	2.4	48.9	2.5	34.1	2.0	37.5	2.5	60.4	2.7	28.6	3.5
CM-F4	68.4	3.2	54.9	3.0	37.4	2.9	40.1	3.0	62.8	3.2	31.0	3.8
CM-A1	71.6	3.4	57.9	3.3	39.3	3.2	41.6	3.1	66.5	3.2	32.4	3.8
CM-A2	67.9	3.6	54.0	3.4	36.8	2.9	39.3	2.8	63.3	3.2	30.0	3.6
CM-A3	72.4	3.4	57.8	3.3	38.4	3.1	42.8	2.9	68.4	3.4	33.1	3.7
CM-A4	70.6	3.3	57.5	3.2	38.9	3.1	42.1	3.3	68.5	3.5	33.1	4.2

to the shaft, although the fusion may not be complete in some adults. According to Baumel & Witmer (1993), the ossified arcus extensorius is also observed in some other taxa. Phylogenetic and functional significances of these ossification centers are not clear, for which further studies are required.

CONCLUDING REMARKS

Comparative study of postnatal ontogeny of major limb bones in five modern birds showed a consistent pattern of bone growth, as well as interspecific variation in the relative timing of skeletal maturation. Longitudinal growth of limb bones generally ceases before or around the time of fledging, coinciding with the ossification of epiphyses. On the other hand, circumferential growth tends to persist after that time, and seems to cease before the time of sexual maturity, although the exact

Table 9. Results of Kruskal-Wallis tests for the difference of skeletal dimensions between fledglings and adults in *Calonectris leucomelas*. For each test, the test statistic H , p -value (p), and the results of the stepwise test procedure of the false discovery rate control (Benjamini & Hochberg, 1995) are shown. For all tests, degree of freedom equals 1.

	H	p	Significance
Length			
Humerus	0.152	0.696	
Ulna	0.343	0.558	
Carpometacarpus	0.060	0.807	
Femur	0.467	0.495	
Tibiotarsus	0.086	0.770	
Tarsometatarsus	0.086	0.770	
Shaft width			
Humerus	7.467	0.006	*
Ulna	6.438	0.011	*
Carpometacarpus	6.705	0.010	*
Femur	0.343	0.558	
Tibiotarsus	3.093	0.079	
Tarsometatarsus	8.029	0.005	*

*: significant at $\alpha = 0.05$ after controlling the false discovery rate for 12 simultaneous tests.

timing is not clear. There can be considerable variation in the degree of maturity (e.g., ossification of epiphyses) among elements even within a single individual, which one should note when dealing with isolated fossils. So far as concerning long bones of modern birds, macroscopic morphological features, such as the morphology of epiphyses and the relative size of foramen nutrien, can be clues to assess ontogenetic stages of specimens, as well as surface textures (Tumarkin-Deratzian *et al.*, 2006; Watanabe & Matsuoka, 2013).

Although problematic in the light of ontogenetic ageing, interspecific variation in absolute and relative rates and timings of skeletal development across various body parts might be of certain evolutionary significance, since dissociation of developments among body parts (Heers & Dial, 2015) may partly mitigate canalization of ontogenetic trajectories due to functional demands in early ontogeny (see Dial, 2003; Dial & Carrier, 2012). Accumulation of more data would shed some light on the role of ontogeny in the evolutionary diversification of birds.

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